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新興病原體之監測與檢體生物材料庫的建立

研 究 報 告

執行機構：國立台灣大學

計畫主持人：陳宜君

研究人員：盧柏樑、盧敏吉、高翠麥、劉佳穎、李官燁、王竣令
胡婉妍、孫幸筠、王振源、廖俊星、盛望徽、王振泰

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＊本研究報告僅供參考，不代表本局意見，如對外研究成果應事先徵求本局同意＊

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摘要

(1) 中文摘要

研究目的：針對住院病人建立整合醫療常規、傳染病通報，提升診斷水準的收案流程，建立有良好臨床及流行病學資料之檢體生物材料庫。

研究方法：在北、中、南三區共四家教學醫院執行，針對臨床上懷疑有感染症，且簽署同意書者，收集病歷資料及臨床檢體。依試驗流程（醫療常規中標準化診斷流程）診治及收集檢體。針對病人基本資料、臨床表現、檢驗結果、治療及預後，研究人員以標準化個案報告單記錄。針對住院病患具臨床徵狀明顯但無法以醫院可提供之臨床檢驗檢測出致病原的疾病，主動收集檢體，建立新興病原之檢體生物材料庫；並依據症候別，利用依單一病原菌 in-house PCR 或多菌套組組的檢測方法(*TrueScience*™ RespiFinder® Pathogen and Viral Identification Panels (Respifinder DC TwoStep kit, Pathofinder, Maastricht, The Netherlands), 以檢測其他可能的致病原（包括 14 RNA viral、1 DNA viral、4 bacterial respiratory pathogen targets)。

主要發現:自 99 年 8 月 18 日至 100 年 6 月 30 日本研究前瞻性收案 138 人，收集 800 檢體，加上 99 年 1 月 1 日至 100 年 6 月 30 日法傳通報者共 522 人，1809 檢體。針對高危險病人群且經照會感染科而送驗之 *Pneumocystis jiroveci* 肺炎借助 PCR 確診高達 40.8% (80/196)，包含 2 confirmed cases、78 probable cases。至於，臨床懷疑退伍軍人病通報個案只有 14.3%(25/175) 確診，針對其中 67 例且有呼吸道檢體的個案加做分子診斷。初步分析顯示(1)臨床鑑別診斷不易，即便在 2009 年 H1N1 新型流感流行期間仍有流感沒有被診斷出來;(2)整合醫療常規、法傳通報及本研究多菌分子診斷,仍有 55.2% 臨床懷疑退伍軍人病通報個案無任何病原菌被檢測出來；(3)呼吸道檢體收集不易且檢體量不足以進行多種檢驗，本研究以收集漱口水克服此瓶頸，

並可偵測出數種病毒。

結論：本研究顯示出醫療常規中病原菌診斷之諸多限制。依現行法定傳染病通報個案之檢體，擬進行新的診斷平台或尋找新病原菌的檢驗，成本效益低。

建議事項：為因應新興病原菌的崛起，擬及時偵測新興病原菌，且有效率的確定及評估新興病原菌與疾病相關性研究的重要元素包括：適當收集、處理及儲存的檢體，良好的臨床及流行病學資料，倫委會通過且檢體於可未來進行其他檢驗，若有適當的對照組檢體會更有價值。

中文關鍵詞： 新興病原菌、分子診斷、法傳通報、退伍軍人病、流感、*Pneumocystis jiroveci*

英文摘要

Objects: to establish the biobank for studying emerging infectious agents

Methods: This multicenter study was conducted at 4 teaching hospitals located in northern, middle and southern Taiwan from January 1, 2010 through June 30, 2011. This study focuses on atypical pneumonia and severe pneumonia with identified etiology and establishes and implements the processes for collection clinical specimens and clinical and epidemiological data from hospitalized patients with clinical evidence of infection. The processes incorporate routine clinical practice and notifiable disease reporting system in order to improve quality of the etiological diagnosis. For those respiratory specimens are collected after patients signed the informed consent and/or specimens were adequate, molecular diagnosis was conducted using either individual, in-house PCR or multipathogen identification panels (*TrueScience*™ RespiFinder® Pathogen and Viral Identification Panels).

Results: From Aug 18, 2010 through June 30, 2011 a total of 800 specimens were prospectively collected from 138 patients who signed the informed consent. There were additional 1009 specimens were sent to CDC and 384 patients were reported to CDC through notifiable disease reporting system. Among 196 high-risk patients who was suspect *Pneumocystis jiroveci* pneumonia following consultation and report to CDC for PCR diagnosis, up to 40.8% were confirmed. For those suspect Legionella disease, only 14.3% (27/175) confirmed the diagnosis. For 67 patients who had adequate respiratory specimens sent for further molecular diagnosis, we identified 3 cases with influenza occurring during 2009 H1N1 influenza pandemic while physicians did not suspect influenza clinically. Despite of these efforts, up to 55.2% of these 67 patients, we did not identify any etiology.

Conclusion: This study showed the limitation of current clinical practice in the etiological diagnosis of atypical pneumonia such as Legionella disease. It might not be a cost-effective strategy if we use specimens collected through notifiable disease reporting system for adapting new diagnostic plateform for emerging pathogens.

Suggestions: Appropriately collected, handled, and stored specimen sets with good clinical and epidmiological data and institutional reiew board approval for future testing will be great assets in efforts to identify and evaluate novel etiology-disease associations. Such specimen sets that also have appropriate controls will be even more valuable; they allow investigators to determine which associations were likely important and worth pursuing.

Key words: emerging infectious agents, notifiable disease reporting system, molecular diagnosis, Legionella disease, influenza, *Pneumocystis jiroveci*

本文

一、 前言

背景與現況：

自從2001年所發生的事件，是10年前無法想像的。從炭疽桿菌作為生物恐怖的工具，West Nile virus，severe acute respiratory syndrome (SARS)疫情，A/H5N1禽流感，到A/N1N1新流感全球疫情，公衛體系及醫療機構皆清楚瞭解必需妥善準備，以因應各種新興或再浮現的傳染性疾病[1]。新興疾病被定義為，新發現或由新的病原菌產生的疾病，在過去20年人類發病率增加的疾病，或在不久的將來將對人類威脅增加[2] ，或者地理分布改變。新興疾病增加的因素相當多元 [3-5]。約有一千種病原菌可在人類產生疾病，包括217種病毒，538種細菌，307種黴菌，66種原蟲等寄生蟲[6]。此外，每種病原菌有若干菌株變異，譬如A族鏈球菌有超過150種菌株。隨著抗藥性菌株及高致病力菌株的崛起，使得目前微生物的檢驗充滿挑戰。即使如此，此豐富的病原菌名單仍無法解釋臨床上許多的疾病。此外，新興傳染疾病的崛起頻率愈來愈快[7]。因此，本計畫擬前瞻性地收集臨床檢體，建立檢驗平台，保留目前病因不明的檢體，以建立新的診斷工具或發現新的病原菌。

研究目的：

建立新興病原之檢體生物材料庫，針對臨床徵狀明顯但無法檢測出致病原的疾病，主動收集檢體。利用新的檢測方法，檢測致病原與尋找新的病原體。致病原與新的病原體的發現，除了提昇台灣傳染病檢驗的能力，帶動生物科技產業的發展。

二、 材料與方法

研究設計：前瞻性，多中心病歷資料及臨床檢體收集。

參與醫院：北中南區共五家教學醫院執行(**表一**)。

收案病人：本計畫收案局限於住院病人，以呈現疾病有一定的嚴重度。病人從 2010 年 5 月（醫院研究倫理委員會通過日起）~2011 年 6 月，成年(16 歲或以上)病人，臨床上懷疑有感染症，且簽署同意書者，將加入此研究。

試驗流程：建立針對住院病人整合醫療常規、傳染病通報，提升診斷水準的收案流程，參**圖一**。針對不明原因肺炎及不明熱之病原菌診斷建立提升診斷水準之流程（參**圖二、三**）。病人依臨床症狀分類，並收集適當部位的檢體（參**表二、表三、圖四**）。依臨床判斷，進行送驗，檢驗項目及送驗單位依各醫院而異。每位病人依醫療常規接受血液檢查、發炎指數、血液及痰液培養、生化檢查、痰液革蘭氏染色及耐酸染色。必要時進行黴漿菌血清檢查、尿液退伍軍人抗原檢查、痰液披衣菌抗原檢查、分子生物學檢查、黴菌抗原血清檢查。部份檢驗非醫療常規，檢體送至台大醫院臨床感染症研究室，以研究計畫經費執行。

資料收集：病人基本資料、臨床表現、檢驗結果、治療及預後，研究人員以標準化個案報告單記錄（**附錄一**）。

定義：各醫院法定傳染病之病例定義，皆依疾管局法定傳染病之收案定義，參**附錄二**。肺炎及病原菌定義參**附錄三**。

檢體收集原則：同醫療常規。若依醫療判定進行法定傳染病通報，則遵守疾病管制局依疾病別訂定之檢體採集規定，包括檢體種類、量及採集時機及頻率（參**表二**）。部分檢驗項目需取得急性期或用藥前之檢體，檢驗方能

檢測出致病原，則需依各醫院之規定，取得檢驗單位之同意，收集三日內送臨床檢驗單位檢驗之剩餘檢體。於第一次收集檢體時，保存部分檢體當作 stock，以備常規檢驗無法確認病原體時，繼續進行其他可能病原體之檢驗或保留供未來檢驗之用。

特殊檢驗：依單一病原菌或群組的實驗室方法及執行單位參**表四**。檢體後續進行之處置及分子檢驗流程參**圖六**。依據症候別，利用依單一病原菌 in-house PCR 或多菌套組組的檢測方法(*TrueScience*™ RespiFinder® Pathogen and Viral Identification Panels (Respifinder DC TwoStep kit, Pathofinder, Maastricht, The Netherlands), 以檢測其他可能的致病原(包括 14 RNA viral、1 DNA viral、4 bacterial respiratory pathogen targets)。

資料分析：描述統計，分析常見病原菌的分布，依地區、依季節，依宿主年齡及診斷分析。

三、 結果

收案情形

本研究包括法定傳染病通報個案回溯性分析及前瞻性收案。各醫院所收集之個案數/檢體數、前瞻性檢體個案數/檢體數、依法定傳染病定義送回本局之個案數/檢體數，參**表五、六、七**。自 99 年 8 月 18 日至 100 年 6 月 30 日本研究前瞻性收案 187 人，收集 798 檢體，加上 98 年 1 月 1 日至 100 年 6 月 30 日法傳通報者共 522 人，1807 檢體。

申請倫理委員會審查期間，以及建立標準診斷流程及可行性測試期間，本研究先針對 2009 年通報疾病中非典型肺炎及不明熱可能通報疾病彙整(結果參**附錄四**)，並以通報退伍軍人病、*Pneumocystis jiroveci* 肺炎或不明原因肺炎送驗疾病管制局保存的痰液檢體進行多種病毒檢測，結果參**附錄五、六、七**。

各醫院個案確診及預後之差異主要來自醫師之臨床經驗及警覺性，有些醫師只針對重症照會感染科醫師，感染科鑑別診斷時考慮該疾病，故進行採檢通報(參**附錄四**)。

退伍軍人病通報個案 (參附錄五)

臨床懷疑退伍軍人病通報個案只有 14.3%(25/175) 確診。針對其中 67 例且有呼吸道檢體的個案加做分子診斷。初步分析顯示臨床鑑別診斷不易，即便在 2009 年 H1N1 新型流感流行期間仍有 4%(3/67)流感沒有被診斷出來。整合醫療常規、法傳通報及多菌分子診斷(CDC in house PCR)，仍有 55.2%臨床懷疑退伍軍人病通報個案無任何病原菌被檢測出來。

Pneumocystis jiroveci 肺炎通報個案 (參附錄六)

針對高危險病人群且經照會感染科鑑別診斷而送驗之 *Pneumocystis*

jiroveci 肺炎借助 PCR 確診高達 40.8% (80/196)，包含 2 confirmed cases、78 probable cases。借助多菌分子診斷(RapidFider) ， 11% (10/91) had additional treatable etiologies identified。

四、 討論

非典型肺炎

社區性肺炎依據臨床表現、形象特徵、流行病學及病因，傳統上區分為典型細菌性肺炎及非典型肺炎，參**表八**之比較。非典型肺炎的病原菌相當多樣，包括細菌(退伍軍人菌、*Mycoplasma* 等)、病毒、分枝桿菌及黴菌(如 *Pneumocystis jiroveci*)，對經驗療法中主要用藥 β-lactam(包含 penicillin 及 cephalosporin)是無效的；但因鑑別診斷及確診不易，且可能引起重症，常是過度使用抗生素或或醫療爭議的重要因素之一。故本研究針對非典型肺炎收案。

前瞻性研究收案 versus 法傳通報系統

由於可能符合收案定義之病人住院單位分散，即時篩選，向病人或家屬說明簽署同意書，並取得適當檢體不易，故依據非典型肺炎醫療常規中會列入鑑別診斷的退伍軍人病、*Pneumocystis jiroveci* 肺炎以及不明原因肺炎，一旦通報至感控單位即進行前瞻性收案。重點單位則拜託主任、主治醫師或相關同仁協助發現適合之個案。總之，相對於法傳通報系統，前瞻性研究收案困難度較高！

本研究發現，某醫院退伍軍人通報個案死亡率很高（參**附錄四**），乃因該院醫療常規中未提供退伍軍人症尿液抗原檢驗，醫師警覺性較低。此等病人皆因重症或對經驗性抗微生物製劑反應不佳，照會感染科醫師，乃通報送驗。故確診率高而死亡率亦高，並非是通報(或收案)定義不同。

呼吸道檢體收集不易

呼吸道檢體收集不易且檢體量不足以進行多種檢驗，本研究以收集漱口水克服此瓶頸，並可偵測出數種病毒。臨床檢體的收集需依據病灶及致病

機轉收集適當的檢體，以提高診斷率（參**表九**）。而非典型肺炎病人若尚未插管，其呼吸道檢體常常不容易取得，其理由包括呼吸道分泌物(痰)少（參**表八**），病人無法配合收集痰液（年紀大、意識改變、品質不良、不配合收集等），而鑑別診斷疾病多，檢驗項目多，常因痰液不足夠進行多種檢查，而導致診斷或研究品質不理想。故本研究依據 2003 年 SARS 研究經驗收集漱口水[18]以克服呼吸道檢體收集之瓶頸。搭配核酸萃取及多菌分子診斷套組，所需檢體量少。初步資料顯示，可由漱口水偵測數種病毒。

特殊檢驗

包括分子生物學研究及病毒培養，依單一病原菌或群組的實驗室方法及執行單位參**表十**。此階段之檢驗評估很重要，應建立檢驗流程，以排除已知之病原菌（**表十一**）。牽涉中心實驗室之檢驗能力、設備，且檢驗成本很高。

近十年重要新興病原菌多屬病毒，而臨床上常忽略此類病原菌之重要性，其理由參**表**。故本研究針對懷疑特定疾病（退伍軍人菌、*Pneumocystis jiroveci* 肺炎）病人之檢體以多菌分子診斷套組偵測非典型肺炎重要病原菌。

病原菌檢出率

臨床懷疑通報個案**診斷率偏低**，且因疾病、醫院而異。**臨床鑑別診斷不易**，即便在 2009 年 H1N1 新型流感流行期間仍有流感臨床沒有懷疑。借助針對**可傳播疾病**之多菌分子診斷，可增加**可治療疾病**之診斷率。

本研究顯示，依照醫療常規進行的檢驗，加上疾管局針對通報疾病進行的檢驗(針對尿液、尿液及血清)，加上針對特定病毒 in-house PCR 分子診斷，仍高達一半的個案沒有任何一種病原菌被檢驗出來。本研究回溯期間包含 2009 年 H1N1 新流感全球大流行，相關宣導很多，疾病管制局也以公務預算提供流感抗原快速檢驗，流感重症病人提供核酸增幅檢測。此研究

發現兩例流感，而臨床上完全沒有懷疑。

目前醫療照護中感染症疾病病原菌判斷的困難參表十三，影響疾病診斷率及病原菌檢出率的因素分析：依病程及檢驗流程探討參表十四，影響疾病診斷的其他議題及建議參表十五 。

五、 結論與建議

有鑑於台灣地區症候群通報系統所暴露之諸多限制，故本計劃著重建立起臨床合作網絡，流程的建立，檢體及資料的收集。建立病例定義，病原菌判斷定義，診斷標準化流程，case report form 及檢體收集。本計劃期間有限，故先針對非典型肺炎及不明原因肺炎重症建立流程，顯示實際可行。未來多年期計畫的重點逐步增加收案族群或疾病別。然而，相對於法定傳染病通報個案數，前瞻性收案及時取得病人之同意書並不容易。

本計劃經費及期程有限，須搭配其他計畫才能進行新的檢驗方法的研發及評估。本研究顯示出醫療常規中病原菌診斷之諸多限制。因此，若依現行法定傳染病通報個案之檢體，擬進行新的診斷平台或尋找新病原菌的檢驗，將耗費成本且很可能徒勞無功！

六、 計畫重要研究成果及具體建議

1.計畫之新發現或新發明

不適用

2.計畫對民眾具教育宣導之成果

不適用

3.計畫對醫藥衛生政策之具體建議

建立並維持臨床合作網絡

為因應新興病原菌的崛起，擬及時偵測新興病原菌，且有效率的確定及評估新興病原菌與疾病相關性研究的重要元素包括下列各點，且須養兵千日以備不時之需！

1. 適當收集、處理及儲存的檢體：參檢體收集及處理流程

2. 良好的臨床及流行病學資料：參 case report form 內容

3. 倫委會通過且檢體可用於未來進行其他檢驗：參倫委會申請文件

臨床試驗/研究受試者說明及同意書部分內容摘錄如下：

四、試驗方法及相關檢驗：依醫療常規，也就是依您的醫師判斷感染症的類型，依標準化的診斷過程，進行採集適當的檢體送驗。檢驗項目及送驗單位依各醫院而異。每位病人依醫療常規及病情之需要採集血液、痰液、尿液作檢驗，只是在上述採檢時，會額外多收集檢體，譬如血液額外多收集 4 毫升、痰液 1 毫升、尿液 20 毫升。特殊情況下您的醫師也可能採集腦脊髓液、肋膜液及肺泡沖洗液等其他檢體接受檢驗，或根據所檢驗的病原菌，於疾病急性期及恢復期各採取一次血清。除了依醫

療常規及病情之需要，本試驗不會增加抽血等收集檢體之頻率，將保存您的部分檢體以進行額外的檢驗項目，尤其是醫療常規下所進行之檢驗無法得知導致您生病之病原菌時。本試驗所採集的檢體及剩餘檢體將保存於台大醫院臨床研究大樓705研究室，保存期限為10年。收集檢體(包括血液、痰液、尿液等)除依醫療常規進行各項檢查，亦會針對病原菌不明者，在本研究中心實驗室（台大醫院臨床研究大樓 705 研究室)或依法定傳染病通報流程由疾病管制局進行額外的檢驗。

五、剩餘檢體處理情形：

若試驗結束後有剩餘之檢體，在您的同意下，台大醫院將保存此檢體，作為未來感染症相關研究之用。所有新的研究計畫都要再經由台大醫院研究倫理委員會審議通過，並於必要時要求重新得到您的同意。剩餘檢體將儲存於台大醫院第 705 研究室超低溫冷凍櫃，檢體保存年限為10 年。

為了保護您的個人隱私，我們將以一個試驗編號來代替您的名字及相關個人資料，以確認您的檢體及與相關資料受到完整保密。在收集後若您有任何想要銷毀檢體的需求，請立即與我們聯絡（聯絡人：XXX 醫師 電話：XXXXXXXX 分機 XXXX；聯絡單位：XXXX 醫院內科部感染科 電話：02-XXXXXXXX 分機 XXXX 地址：XXXXXXXXXXX)，並授權 XX 醫院研究倫理委員會審議是否需要再取得您的同意：

4. 若有適當的對照組檢體會更有價值：需依據臨床問題，設定或選擇對照組。以社區為主兒童急性呼吸道感染症研究而言，需依據年齡層及季節 match 的無症狀的、健康兒童對照組。本研究無對照組檢體，故若擬進一步探討檢測出之病菌之臨床意義，有其限制。

建立新興病原菌研究策略

1. 本計劃規劃之多中心合作模式，針對非典型肺炎及不明原因肺炎重症監測系統、通報流程與檢體生物材料庫之建立，可為日後推廣相關疾病監測送驗流程之參考。
2. 但是，此監測依賴人力以維持收案，及依賴經費以進行醫療常規中無法提供之檢驗。此外，不明熱之收案流程之可行性尚待克服。
3. 如何在有限的樣品下，尋找其中已知或未知的病原體。以大海撈針的方式尋找病原體，常面臨檢體不足，或因為犧牲敏感性而一無所穫。應依症候群或宿主等特性，建立檢測流程。
4. 新的診斷平台或尋找新病原菌的檢驗昂貴，因此如何篩選適合的檢體進行前測或詳細研究，成為此等研究符合成本效益的關鍵因素。

建立病原菌與疾病相關性研究策略

1. 許多已知病原菌與疾病相關性研究有限，且尚待定期逐一檢視或更新病原菌定義。
2. 須長期栽培相關研究團隊，並依據 21 世紀的柯霍假說（參**表 16**），建立病原菌與疾病相關性研究策略。

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八、 圖、表

表一、參與研究計畫之教學醫院

研究醫院	科部	研究人員
國立台灣大學醫學院附設醫院	感染科	陳宜君主任、高翠麥醫師、李官燁醫師、胡婉妍醫師、孫幸筠醫師、孔祥琪醫師、盛望徽醫師、洪健清醫師、王振泰醫師等
	胸腔科	王振源醫師、蔡子修醫師、許嘉林醫師等
	加護病房	柯文哲主任
亞東紀念醫院	感染科	廖俊星主任、劉佳穎醫師
中山醫學大學附設醫院	感染科	盧敏吉主任
高雄醫學大學附設中和紀念醫院	感染科	盧柏樑主任
財團法人義大醫院	感染科	林錫勳主任、王竣令醫師

義大因助理聘任困難，故無法繼續參與前瞻性計畫

表二、疾病管制局肺炎檢驗項目及檢體採集注意事項

病源菌	檢體種類	採檢目的	採檢時機	採檢規定	運送條件	注意事項
Legionella 退伍軍人病	痰液、呼吸道分泌物、胸膜液	病源體檢測(分離、鑑定)	立即採檢	以無菌容器收集直接嗽出之痰液。	低溫	1.勿以棉花拭子採集痰液、呼吸道分泌物、胸膜液等檢體。2.勿採患者口水。3.痰液檢體採檢請參考第 3.9 節。4.胸膜液等體液採檢請參考第 3.10 節。
	尿液	病源體檢測		以無菌容器收集 10mL 尿液。		尿液檢體見 2.7.2 備註說明，尿液檢體採檢步驟請參考第 3.4 節。
	血清	抗體檢驗(IFA)		以無菌試管收集至少 3mL 血清。		血清檢體見 2.7.3 及 2.7.4 備註說明，血清採檢步驟請參考第 3.3 節。
Leptospira 鉤端螺旋體病	尿液	病源體檢測(分離)	發病 10 天後，且未投藥前	以無菌容器收集 10mL 中段尿液。並添加 0.5mL 之 1M 磷酸緩衝液 (phosphate buffer) (pH 7.4)。	低溫	
	抗凝固全血	病源體檢測(分離)	高熱期，(發病 10 天內且未投藥前)	以含抗凝劑 (EDTA) 採血管採集 5mL 血液檢體，並混	常溫	

				合均勻。		
	血清	抗體檢驗 (MAT)	發病 8-14 天之間	以無菌試管收集 3mL 血清。	低溫	
	腦脊髓液	病源體檢測(分離)	具無菌性腦膜炎症狀，發病 5-10 天之間	以無菌檢體小瓶收集 0.5 mL 腦脊髓液。	常溫	
Pneumocystis jiroveci 肺炎/ CMV	痰液					
流感	鼻腔拭子、喉嚨拭子、痰液					
	血清					

表三、病人依臨床症狀分類，並收集適當部位的檢體

症候群	血液檢體	尿液檢體	呼吸道檢體	腦脊髓液	關節液	腸道檢體
肺炎	全血 血清	V	痰液、漱口水、肺部沖洗液 (optimal)			
不明熱、敗血症	全血 血清	V				
腹瀉						V
腦炎、腦膜炎	血清			V		
關節炎	血清				V	
發燒及皮疹	全血 血清					

說明：

[1] 血液檢體： CBC 管 3ml, 生化管 3ml

[2] 呼吸道檢體：nasopharyngeal swab (for influenza), throat swab, sputum, endotracheal aspirate (prefer), bronchoalveolar lavage[8]（參圖三說明）

[3] 腸道檢體：糞便、肛門拭子

表四、依單一病原菌或群組的實驗室方法及執行單位

病原菌	檢體	實驗室方法	執行單位	說明或依據
Influenza A/H1/H3/swH1 viruses Influenza B RSV-A/B Adenoviruses Human metapneumovirus Rhinoviruses HSV1 HSV2 CMV parainflunz type1 parainfluenza type 2 parainfluena type 3 Enterovirus	Sputum	RT-PCR	疾管局劉銘燦博士	Ward, et al (2004). J Clin Virol 29, 179-188 ; Yang JR, et al. (2009) J Clin Microbiol 47: 3714-3716; Suwannakar, K. et al. (2008). J Virol Methods 152,25-31. Ward, et al (2004). J Clin Virol 29, 179-188 Bonzel, L. et al., (2008). Pediatr Infect Dis J 27, 589-594. Maertzdorf, J. et al. (2004). J Clin Microbiol 42, 981-986. Karatas, H. et al. (2008). . J Neurol Sci 264, 151-156. Bonzel, L. et al. (2008).Pediatr Infect Dis J 27, 589-594.
Legionella	Sputum, urine		疾管局江春雪博士	
Pneumocystis jiroveci	Sputum		疾管局嵇達德博士	
Fungi	Sputum,		台大醫院、疾	

	gargling, blood	管局李淑英博士	
Simultaneous detection of 15 common viruses and 4 bacterial respiratory pathogen from respiratory samples was conducted using a multiplex ligation-dependant probe amplification (MLPA) (*TrueScience*™ RespiFinder® 19 Pathogen and Viral Identification Panels, Pathofinder, Maastricht, The Netherlands) for Influenza A, Influenza A H5N1, Influenza B, RSV A, RSV B, Adenovirus, Human Metapneumovirus, Rhinovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Coronavirus 229E, Coronavirus NL63, Coronavirus OC43, *Bordetella pertussis, Chlamydophila pneumonia, Mycoplasma pneumonia, Legionella pneumophila.*	Sputum, mouth wash	台大醫院	Currently, there are >200 known respiratory virues, but accurate data on how many community-acqureid pneumonia cases are caused by viral pathogens are lacking or limited. This choice in the panel is in conjunction with the epidemiological data (Clin Infect Dis 2007;44:S27; Clin Infect Dis 2008;47:S123) Limitation: serology and viral culture not conducted in this study due to cost constraint. The assay includes a combine reverse transcription/PCR step, a probe hybridization step, and a probe ligation/amplification step. An internal amplification control is included in the sasay to distinguish beween true negative samples and false negatives cuased by nucleic acid degradation, PR inhibition, or handling errors.The MLPA was conducted according to the manufacturers' protocol using an ABI2720 StepOne thermocycler (Applied Biosystems, Foster City, CA, USA).

表五、收案情形

個案數	總計	台大	亞東	高醫	中山	義大[1]
總數[2]	522	311	85	118	4	5
通報個案數(98.1.1~98.12.31)	69	32	9	23	0	5
通報個案數(99.1.1~100.6.30)	314	246	76	76	1	0
非通報個案數(99.1.1~100.6.30)	38	33	0	19	3	0
簽署同意書個案數(99.8.18~100.6.30)[3]	187	153	3	27	4	0
CRF 完成數	411	228	85	89	4	5

表六、檢體收集情形（99 年 1 月 1 日至 100 年 6 月 30 日檢體總數 1595）

檢體數	總計	台大	亞東	高醫	中山	義大[1]
總數	1807	1130	292	353	19	13
CDC 檢體數(98.1.1~98.12.31)	212	95	25	79	0	13
CDC 檢體數(99.1.1~100. 6.30)	797	359	255	180	3	0
簽署同意書核心實驗室收集之檢體數	798	676	12	94	16	0

[1] 義大因助理聘任困難，故無法繼續參與前瞻性計畫

[2] 一個人可能重複被收案或通報不只一個疾病

[3] 簽署同意書病人可能也同時通報並送驗到 CDC

表七、核心實驗室檢體收集進度－依疾病分類（99 年 8 月 18 日-100 年 6 月 30 日）

疾病分類	個案數[1]	檢體數	全血	血清	呼吸道	CSF
肺炎						
退伍軍人症	24	85	40	41	2	2
Pneumocystis jiroveci 肺炎	100	308	145	149	14	
不明原因肺炎	18	93	37	37	19	
病毒						
不明熱	14	30	13	12	4	1
血腫病人[2]	22	249	83	77	89	
其他	9	33	12	12	7	2
總數	187	798	330	328	135	5

[1] 同時通報退伍軍人症及 PJP 之個案，其檢體數算在退伍軍人症下

同時通報 *Pneumocystis jiroveci* 肺炎及不明原因肺炎之個案，其檢體數算在 PJP 下

同時通報 *Pneumocystis jiroveci* 肺炎及懷疑黴菌（血腫）之個案，其檢體數算在黴菌（血腫）下

[2] Persistent febrile neutropenia despite of adequate antibacterial therapy in high risk patients

Table 8. Comparison of atypical pneumonia and common bacterial pneumonia

	Atypical	Bacteria
Etiology	*Mycoplasma pneumonia, Chlamydophila pneumonia, Legionella* species, *Influenza A* (H3N2, pH1N1, H5N1), severe acute respiratory syndrome (SARS) coronavirus, varicella, other respiratory viruses	*Streptococcus pneuoniae, Haemophilus influenza,*
Epidemiology	Epidemic	Not relevant
Clinical findings		
Onset	Preceding URI; malaise; gradual development	Variable URI; constitutional symptoms; More acute onset
Type of cough	Nonproductive or scant (mucoid)	Purulent sputum (bloody)
Pleuritic pain	Uncommon	Common
Fever	Uncommon	Common
Chills	Low to moderate grade	High
Extrapulmonary symptoms	More common	Less common
Physical findings		
Tachycardia	Not striking	may up to 120/min
Physical examination of chest	Modest findings	Consolidation (dullness), egophony, rales
Image		
Pleural effusion	Rare	More common; empyema
Microbiological testing		
Sputum smear	PMN, mononuclear cells	PMN and intracellular bacteria

Blood culture	Negative	May be positive
Serology	Useful	Not helpful

Table 9. Sampling strategies according to the knowledge of pathogenesis of infectious diseases, invasive pulmonary aspergillosis as an example

Pathogenesis	Sampling strategies
Inhalation	Sputum
Respiratory tract (sinus and lung): tissue invasion and damage	Bronchoalveolar lavage (more central localization, endobrochial distribution), fine needle aspiration, or open biopsy (subpleural localization)
Blood: dissemination to non-contiguous sites (liver, spleen, kidney, CNS…….)	Blood (and/or metastatic foci)

Reference: Hope et al. Lancet Infect Dis 2005; 5: 609–22

Table 10. Comparison of several multipathogen panels.

This study focuses on acute respiratory tract infection. Acute respiratory disease accounts for an estimated 75% of all acute morbidities in developed countries, and most of these infections (approximately 80% are viral (Clin Microbiol Rev 2008;21:716). In clinical practice, a specific virus is often not identified due to the lack of sensitive tests and /or the presence of as-yet-unknown pathogens (J Clin vitrol 2007;40:S24; Clin Infect Dis 2007;44:904).

	Life Technologies RespiFinder® 19	Seegene Seeplex RV/PB18 ASE	Abbott xTAG RVP	Abbott xTAG RVP FAST	Qiagen ResPlex II
	CE IVD	CE IVD	CE IVD	CE IVD	RUO
Influenza A (nonspecific)	x	x	x		x
Influenza H1, H3 (subtype)			x	x	H1N1 variant 2009
Influenza H5	x		x		
Influenza B	x	x	x	x	x
Respiratory Syncytial Virus (RSV) A	x	x	x	x	x
Respiratory Syncytial Virus (RSV) B	x	x	x	x	x
Parainfluenza 1, 2, 3, 4	x	x	x	x	x
Adenovirus	x	x	x	x	x
Human Metapneumovirus	x	important missing!	x	x	x
Rhinovirus/Enterovirus	x	x	x	x	x
Coronavirus NL 63, 229E, OC43	x	x	x	x	x
Coronavirus HKU1			x	x	
Coronavirus SARS			x		
Bocavirus		x		x	x
Coxsackievirus					
Echovirus					
Bordetella pertussis	x	important missing!			
Chlamydophila pneumoniae	x	x			
Legionella pneumophila	x	x			
Mycoplasma pneumoniae	x	x			
Haemophilus influenzae	x	x			
Streptococcus pneumoniae		x			

Table 11. Viral etiologies of upper and lower respiratory tract infections

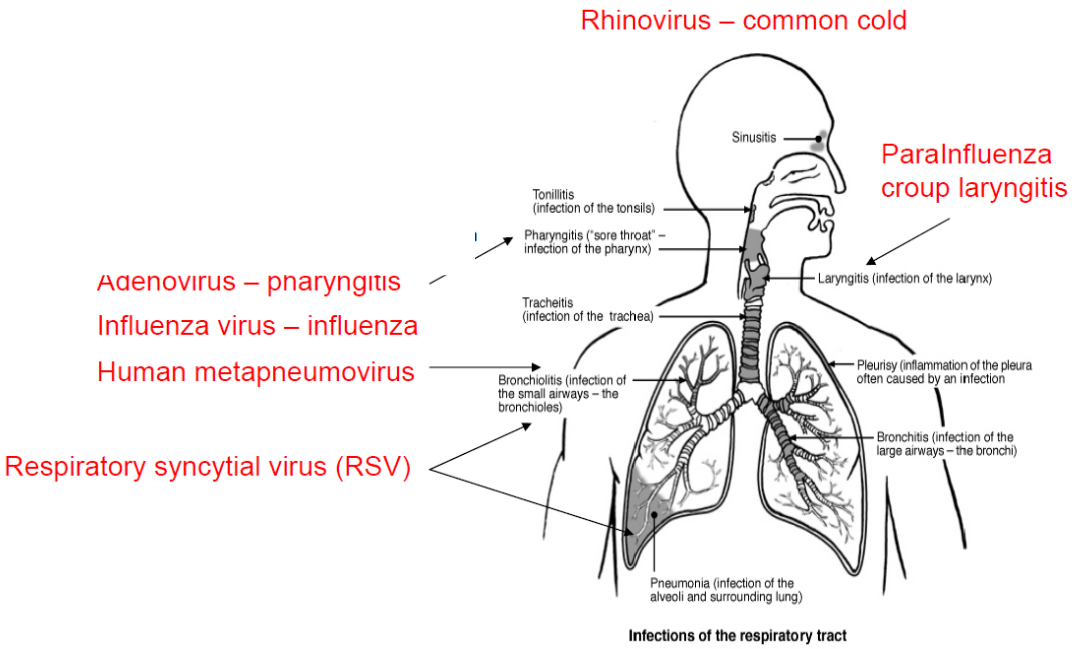
Symdrome	Viral etiologies
Common cold (Coryza)	Respiratory syncytial virus (RSV)*
	Parainfluenza 1 and 2
	Echo 28
	Coxsackie A21, A24
	Rhinovirus (80+ serotypes)
Pharyngitis	Adenovirus (Adenovirus serotype 40*)(MMWR 2007;56:1181)
Pharyngoconjunctivitis	Influenza*, parainfluenza (1-5)
	Coxsackie, Echo
Influenza	Influenza A, B, C
Herpangina	Coxsackie A
Laryngotracheobronchitis (Croup)	Parainfluenza 1 and 2
	RSV, adenovirus

*Progression to or presenting as lower respiratory tract infection.

Table 12. The most common etiological agens of community-acqureid pneumonia in different tpes of patients

Patient type	Etiology
Outpatient	*Streptococcus pneuoniae, Mycoplasma pneumonia, Haemophilus influenza, Chlamydophila pneumonia,* respiratory viruses
Inpatietns, non-ICU	*Streptococcus pneuoniae, Mycoplasma pneumonia, Chlamydophila pneumonia, Haemophilus influenza, Legionella* species, aspiration, respiratory viruses
Inpatients, ICU	*Streptococcus pneuoniae, Staphylococcus aureus, Legionella* species, gram-negative bacilli, *Haemophilus influenza,Influenza A* (H3N2, pH1N1, H5N1) *,severe acute respiratory syndrome (SARS) coronavirus,*

Adapted from Clin Infect Dis 2007;44:S27; Clin Infec Dis 2008;47:S123



表十三、目前醫療照護中感染症疾病病原菌判斷的困難

病原菌	說明
常見細菌	1. 菌量低 (adult versus child)。 2. 已使用有效的抗細菌藥物(such as community-acquired pneumonia due to *Streptococcus pneumonia*, endocarditis cause by viridians streptococci)。 3. 檢體採集的時間(包括血球培養、尿液肺炎球菌抗原檢測等。譬如，敗血症病人於寒顫或高燒時採集的血液培養陽性率較高)。 4. fastidious pathogen such as *Neisseria gonorrhea*，需適當transport medium，或立即檢體處理(包括培養)。
非典型細菌	已知病原菌傳統檢驗方法的限制： 1. 以例行培養基常無法培養出來(such as *Legionella, Helicobacter*)。 2. 依賴特殊抗原或抗體進行血清學檢驗方法(such as *Mycoplasma, Chlamydia, Legionella*)。
病毒	1. 臨床警覺度低，需特殊培養，且許多醫院檢驗單位沒有提供服務。Such influenza virus, enterovirus, adenovirus, etc. 2. 許多傳統已知病原菌的檢驗只有若干大型醫院提供，且價格偏高 3. 依國內建保給付及醫療服務型態，使用限制多。Such as CMV, EBV, parvovirus. 4. 限少數研究單位才有提供，且受限於經費，非例行接受檢體。Such as dengue virus, pavovirus, norovirus, etc. 5. Cell culture and immunoassays to detect the 7 “usual suspects”: influenza viruses A and B; parainfluenza viruese 1,2,3; respiratoyr syncytial virus; and adenovirus (Clin Infect Dis 2008;47:S123)
黴菌	1. 侵襲性黴菌感染因疾病及病人特性，加上上述原因，病原菌確診之比例很低。

	2. 菌量低
分枝桿菌	1. 臨床警覺度低，需特殊培養，且許多醫院檢驗單位沒有提供服務。 2. 菌量低
輸入性病原菌	限政府研究單位才有提供。
新興病原菌	需臨床警覺，結合微生物及生物科技，建構新的診斷工具。

表十四、影響疾病診斷率及病原菌檢出率的因素分析：依病程及檢驗流程探討

步驟	因素	範例及建議
臨床診斷	臨床警覺	關鍵因素
	鑑別診斷	及早照會相關醫師協助鑑別診斷，並依據診斷流程適切採檢送驗，以提高已知病原菌的檢出率，同理，應會提高未來新興病原菌檢出機會。
通病疾病定義	通病疾病定義不足以涵蓋所有血清型	腸病毒重症通病疾病定義不足以涵蓋所有血清型。
	流行病學	EV71 與克沙奇 B3 病毒臨床表現、主要年齡層分布不同。
採檢時間	病程	1 依據致病機轉決定檢體部位及最適採檢時間 1.1 流感病毒量在發病（發燒）D1～4 最高，發展為重症（4 天）後鼻咽檢出率偏低。轉診重症病人收集檢體時已發展至急性呼吸窘迫症候群（ARDS）流感病毒量檢出率偏低 1.2 SARS 病毒量在發病（發燒）D7～10 最高。 1.3 血清抗體產生一般是 2 週，但退伍軍人病可能延遲到 6 週。 1.4 一般而言，在免疫不全病人血清抗體可能延後且陽性率較低。 2 已使用有效的抗微生物製劑影響檢驗結果 2.1 培養：community-acquired pneumonia due to *Streptococcus pneumonia*, endocarditis cause by viridians streptococci 2.2 抗原檢測：Prior use of anti-mold agent and yield rate of galactomanna antigen assay 3 解決方法 3.1 不論疾病嚴重度皆收案，以收集在病程初期的個案。

		3.2 提早收案，只要是曾就醫病人可直接收案。 3.3 收案最初 4 天連續收集呼吸道及尿液檢體。 3.4 血清檢體收案前、收案時，病程 2 週，病程 6 週。
檢體部位選擇		1 依據致病機轉決定檢體部位（及最適時機） 2 流感病毒輕症病人鼻咽拭子(需用指定的病毒採檢拭子)拭子及痰液高，重症病人下呼吸道檢體（endotracheal aspirate）檢出率（及病毒量）較上呼吸道檢體高 3 Feasibility of sampling 3.1 high drop out rate for three samples) (Int J Tuberc Lung Dis 2000;4:246-51; Int J Tuberc Lung Dis 2002;6:222–30) 3.2 Brochoscope and biopsy in critically ill patients 3.3 For patients with complete sampling, a microiological agent was identified for 89% of the cases (Clin Infect Dis 2010;50:202). 4 Nucleic acid amplification testing for tuberculosis not work with pleural effusions and other nonrespiratory samples because of inhibitors in pleural fluids (*Eur Respir J 2003; 21:220-24; Chest 2007; 131:1133-41*)
採檢方法		流感病毒抗原快速篩檢試劑專用拭子
檢體量		呼吸道結核建議取三套痰液，且晨間留取 (Int J Tuberc Lung Dis 2000;4:246-51; Int J Tuberc Lung Dis 2002;6:222–30) 結核性腦膜炎腦脊髓液檢體量傳統建議是 30mL，現醫療常規皆忽略故診斷率很低 若低菌量（1-10 per mL），一般分生檢驗之檢體量（100 uL）建議則不適用
檢體處理	Physical processing method	Sedimentation or centrifugation: concentrate (centrifuge) fluids for culture (centrifuged any sterile fluid over 2 ml and used the sediment for culture)

	Chemical processing method	Liquefaction of sputum for TB with NaOH, NaOCl, NaLC-NaOH (*Lancet Infect Dis 2006;6:664*). Pre-treat respiratory samples for virus using dithiotreitol (DTT, 10% v/v) if specimens are viscous and/or contain solid cellular compoentns (according to RespiFinder handbook).
	核酸萃取	退伍軍人病痰液抽取，DNA Roche 比 BioNeumeric 偽陽性低（江博士）
檢驗項目	鑑別診斷	如何在有限檢體做出病原菌，如何兼顧成本效期。如何建立個人化標準醫療診斷流程。 可能病原菌之種類（細菌、病毒）
	醫療常規與健保給付	醫院檢驗室未提供之檢查，或容易被健保核刪，或自費項目。
檢測方法		1 Limitation of traditional microbiological methods for detection of respiratory tract pathgoens (Cln Infect Dis 2008;47(Suppl 3):S123) 1.1 Viral culture (gold standard, slow) 1.2 Direct fluorescent antibody (DFA) rapid tests (not very sensitive) 2 Opportunity and limitation of molecular diagnostic tests for common and atypical causative pathogens 2.1 Often alck standardization and are not widely available 2.2 Targeted PCR (sensitive, but only one or two targets per test) versus multipathogens panel approach 2.3 Nucleic acid-amplification methods: real-time amplification systems, multiplex analysis, and liquid –bead arrays (Clin Microbiol Rev 2008;21:716-747) 2.4 Others: microarray technology, consensus PCR assays, and high-rhroughput sequencing, such as high-density picrolitre reactors (Nature 2005;437:376)
檢驗試劑		流感病毒抗原快速篩檢試劑：以廠牌(QuickVue A+B, Quidel, San Diego, CA, USA)陽性率最高

A prospective study in Sweden found that a microbial etiolgoy could be identified for 67% of

the patients with community-acqured panumonia by supplementing traditional diagnostic methods with PCR-based methods (Clin Infect Dis 2010;50:202). For patients with complete sampling, a microiological agent was identified for 89% of the cases. Te hmost frequently detected pathgoens were S. pneumoniae (38%) and respiratro yvirus (29%). Two or more pathgoens were present in 35% of cases wit ha determined etiology.

表十五、影響疾病診斷的其他議題及建議

判讀標準	1.	Definition of smear positive cases（Int J Tuberc Lung Dis 2007;11:953–58）
	2.	Cut-off value: Analyses of sensitivity and specificity assume that the cut-off separating categories has already been decided. Often the cutoff is not obvious and is decided as a compromised between sensitivity and specificity. A receiver operating curve (ROC) can help decide the optimal cutoff. The appearance of the ROC and choice of optimal cut-off will vary with the incidence of disease in the population under study (pretest probability) (Clin Infect Dis 2003;36(Suppl 3):S123; Thorax. 2006;61:783; MMWR 2009;58:7-10; JAMA 2000;283:639-45, Chest 2007;132:946-51; Health Technol Assess. 2007;11:1-196, JRCCM1996;153:1606-10; Thorax 1996;51:320-22, Clin Infect Dis 1996;23:1099-1106
臨床意義判讀	1	Diagnostic criteria vary by etiology (Clin Infect Dis 2010;50:202)
	2	被檢測出的病原菌不一定是該病人感染症之病原菌
	3	不只一種病原菌存在時的臨床意義 (J Clin Microbiol 2008;46:97; Clin Infect Dis 2010;50:202)
	4	How to determine what diseases novel pathgoens cause (Clin Infect Dis 2007;44:911). Time-modified Koch's postulates for establishing a causal link between a pathogen and disease include (1) consistenly finding the pathogen in patients with the disease more often than in control subjects, (2) replicating the disease after challenging an appropriate animal with the pathogen, and (3) reisolating the pathogen fro mteh challenged ill animal. A causal relationship is also supported by demonstration of the pathogen in affected tissue (especially histologically), demonstration fo an immune response to the pathogen, and preventi onfo disease with a specific intervention, such as immune therapy or vaccination. Human bocavirus (HBoV) is a newly identified virus tentatively assigned to the family Parvoviridae, subfamily Parvovirinae, genus Bocavirus. HBoV was first described in 2005 and has since been detected in respiratory tract secretions worldwide. A characteristic feature of HBoV studies is the high frequency of coinciding detections, or codetections, with other viruses. Available data nevertheless indicate a statistical association between HBoV and acute respiratory tract disease. We present a model incorporating these somewhat contradictory findings and suggest that primary HBoV infection causes respiratory tract symptoms which can be followed by prolonged low-level virus

		shedding in the respiratory tract. Detection of the virus in this phase will be facilitated by other infections, either simply via increased sample cell count or via reactivation of HBoV, leading to an increased detection frequency of HBoV during other virus infections. We conclude that the majority of available HBoV studies are limited by the sole use of PCR diagnostics on respiratory tract secretions, addressing virus prevalence but not disease association. (Clin Microbiol Rev 2008;21:291-304)
其他 Suggestion	1.	Appropriately collected, handled, and stored specimen sets with good clinical and epidmiological data and institutional reiew board approval for future testing will be great assets in efforts to identify and evaluate novel virus-disease associations. Such specimen sets that also have appropriate controls will be even more valuable; they allow investigators to determine which associations were likely important and worth pursuing. (Clin Infect Dis 2007;44:911)
	2.	Trials of diagnostic tests must define the population appropriate for testing and the clinical question being asked (Clin Infect Dis 2003;36(Suppl 3):S123). The unanswered question for the assay is whether knowledge of results can change the use of antimicrobial therapy, or implementation of infection control measures, or make decision or policy. The impact or clinical implementation of the test resutls might differ according to the incidence of disease in the population under study and clinical course or outcome without any intervervention.

Table 16. Koch's postulates for the 21st century

1. A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology.

2. Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease.

3. With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur.

4. When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship.

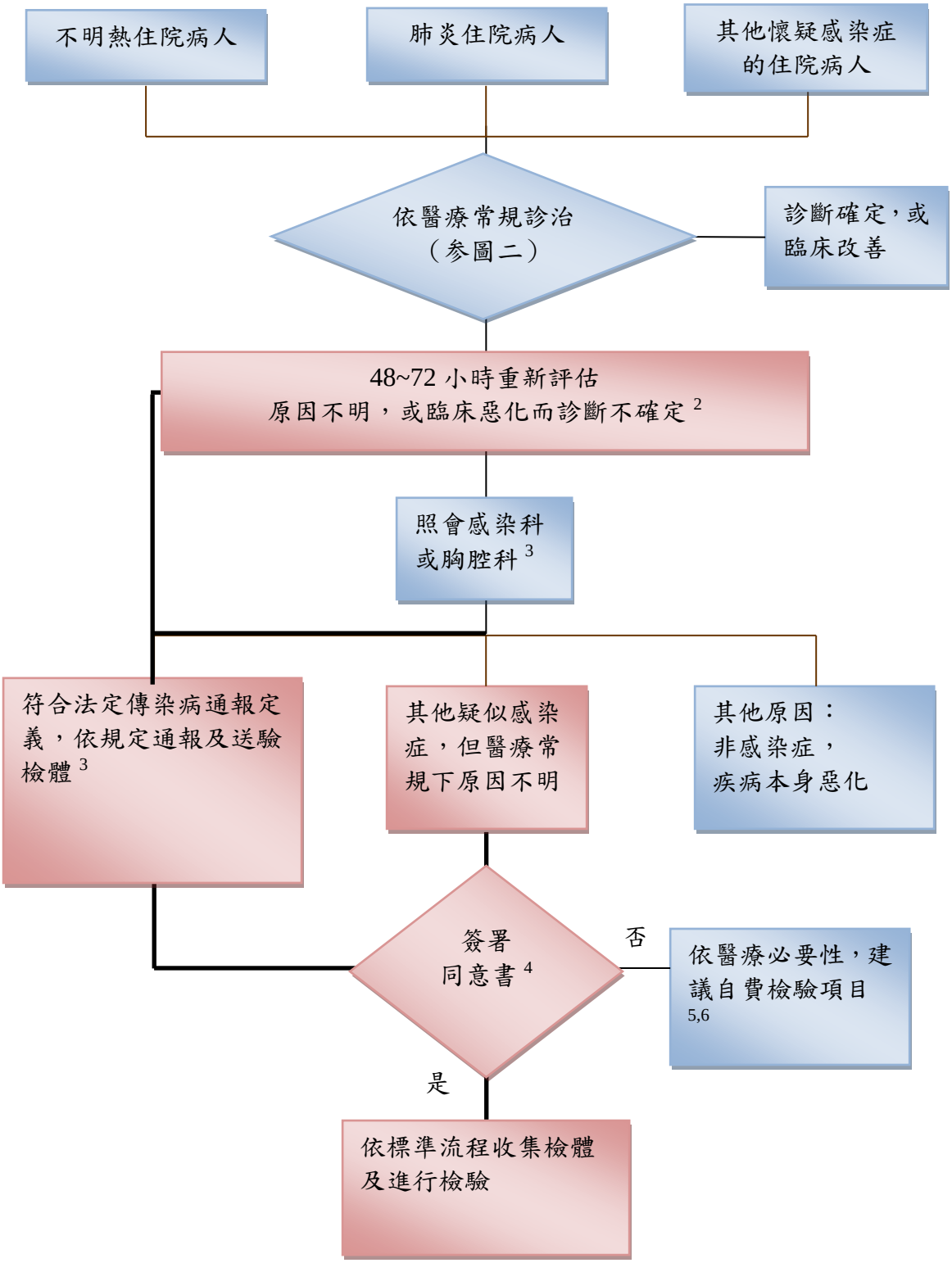
5. The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms.

6. Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located.

7. These sequence-based forms of evidence for microbial causation should be reproducible.

Reference: Fredericks DN, Relman DA (1996). "Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates". *Clin Microbiol Rev* 9 (1): 18–33.

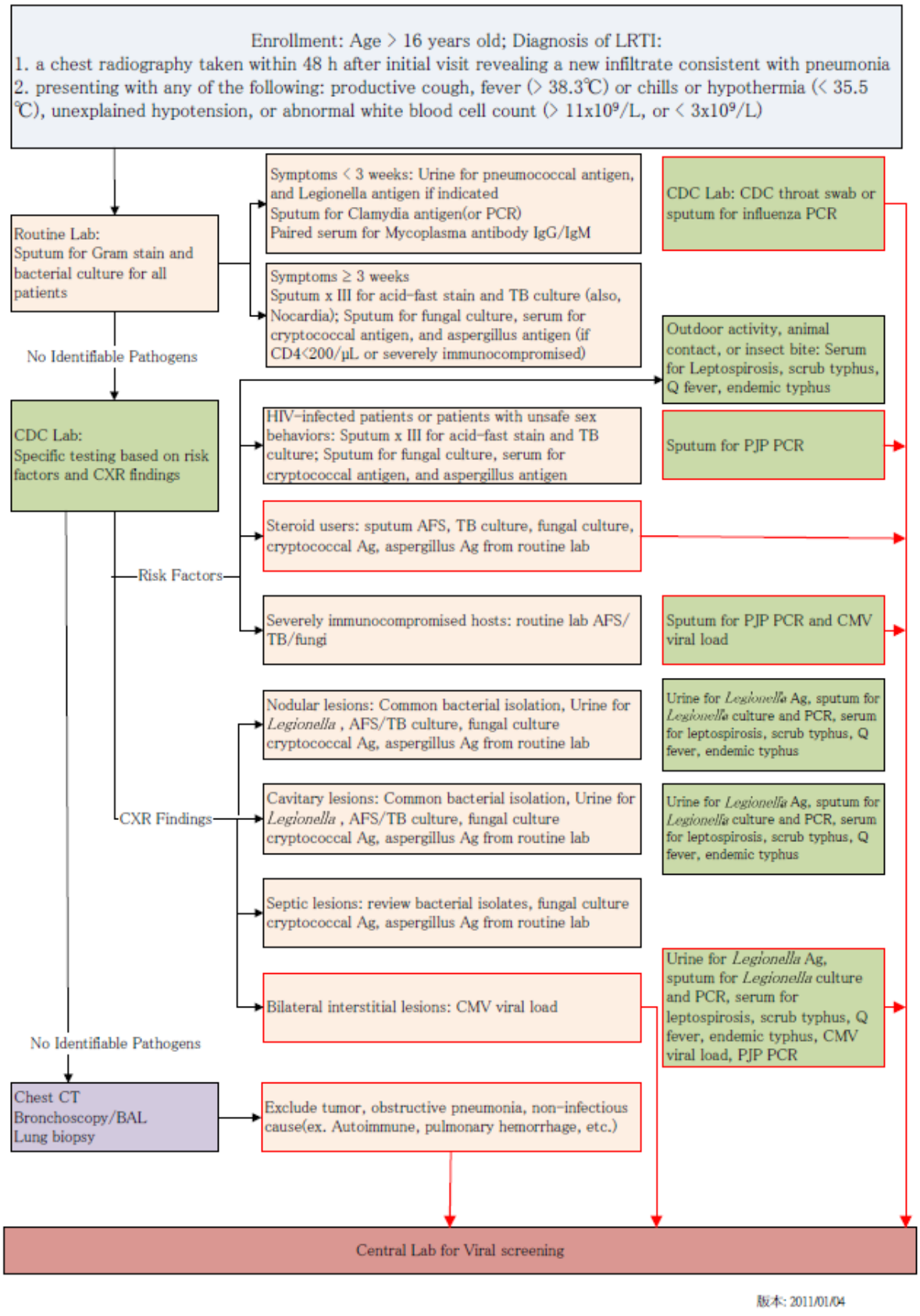
圖一、住院病人整合醫療常規、傳染病通報，提升診斷水準的收案流程[1]



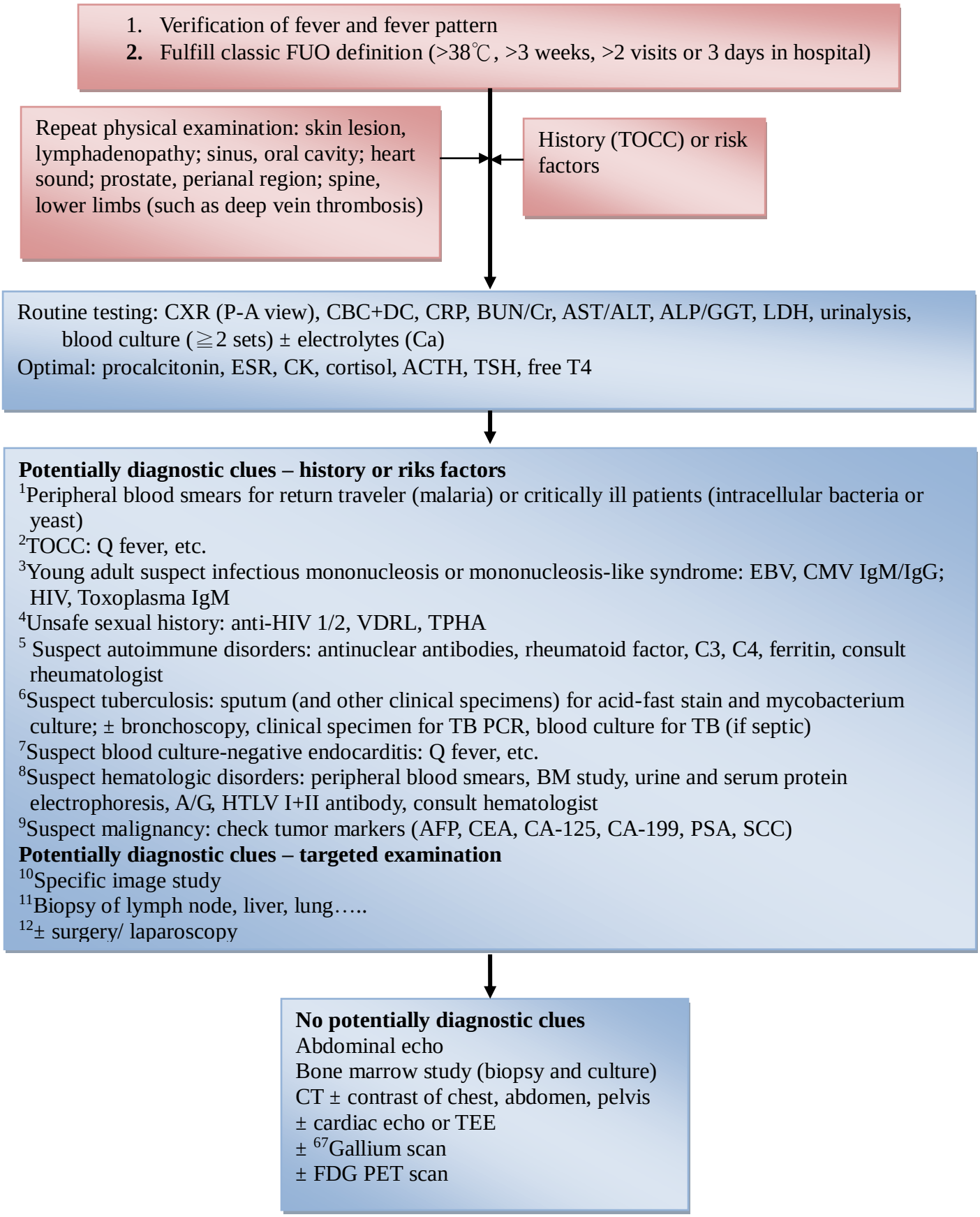
說明：

1. 定點醫師呼吸道感染監測系統可彌補**本研究之不足**。本研究僅針對住院病人，希望能發現會引起相當疾病嚴重度之新興病原菌。本研究著重急性感染症，病程超過一個月的個案不納入，因結核菌及其他分枝桿菌感染的機率增加，且急性感染症病原菌檢出率極低。
2. **診斷不確定**，包括
   (1) 僅符合該病原菌病例定義懷疑（suspect）或可能（possible）個案，未符合極可能（probable）或確定（definite 或 proven）個案。
   (2) 符合某病原菌之極可能或確定病例定義，但部份臨床表現或病程變化無法以該病原菌解釋。適用於有 co-pathogen 之情形，如原本健康者罹患流感重症合併肺炎球菌侵襲性感染，免疫不全病人罹患肺炎球菌侵襲性感染及黴菌肺炎。
3. **照會感染科或胸腔科**：
   (1) 曾就醫病人，或轉院病人之住院初次評估即適用。
   (2) 48~72 小時重新評估，原因不明或臨床惡化（病況惡化者包含急性呼吸窘迫症候群）而診斷不確定病人。
4. **本研究之執行以「依法通報及送檢」為最優先考慮，簽署同意書為輔**：
   (1) 為了維護傳染病通報之完整性，避免因短期研究影響通報系統之代表性，並藉由本研究提升通報系統之功能，且以及彌補「臨床試驗需取得病人或法定代理人的簽署同意」而影響收案病人在母群體之代表性之限制，故本研究之執行以「依法通報及送檢」為最優先考慮。
   (2) 輔以簽署同意書，以彌補不符合通報傳染病定義之個案可納入本研究。
5. **醫療常規之診治品質差距很大：**
   (1) 各醫院可提供檢驗項目不同，且部分是自費項目，故醫療常規中執行不一致，影響診斷品質很大。
   (2) 醫療常規之診治品質各醫院差距很大，本研究針對退伍軍人症分析即顯示此差異。雖然皆依據疾病管制局通報定義通報，但某醫院醫師警覺性較低，只有在重症或病況惡化時才照會感染科醫師，且該院未提供尿液退伍軍人菌抗原檢驗，感染科建議下通報及送驗，故死亡率高。
   (3) 醫療常規之診治品質因醫師而異。

圖二、不明原因肺炎診斷流程



圖三、不明熱診斷流程圖


1. Verification of fever and fever pattern
2. Fulfill classic FUO definition (>38℃, >3 weeks, >2 visits or 3 days in hospital)
Repeat physical examination: skin lesion, lymphadenopathy; sinus, oral cavity; heart sound; prostate, perianal region; spine, lower limbs (such as deep vein thrombosis)
History (TOCC) or risk factors
Routine testing: CXR (P-A view), CBC+DC, CRP, BUN/Cr, AST/ALT, ALP/GGT, LDH, urinalysis, blood culture (≧2 sets) ± electrolytes (Ca)
Optimal: procalcitonin, ESR, CK, cortisol, ACTH, TSH, free T4
Potentially diagnostic clues – history or riks factors
[1]Peripheral blood smears for return traveler (malaria) or critically ill patients (intracellular bacteria or yeast)
[2]TOCC: Q fever, etc.
[3]Young adult suspect infectious mononucleosis or mononucleosis-like syndrome: EBV, CMV IgM/IgG; HIV, Toxoplasma IgM
[4]Unsafe sexual history: anti-HIV 1/2, VDRL, TPHA
[5] Suspect autoimmune disorders: antinuclear antibodies, rheumatoid factor, C3, C4, ferritin, consult rheumatologist
[6]Suspect tuberculosis: sputum (and other clinical specimens) for acid-fast stain and mycobacterium culture; ± bronchoscopy, clinical specimen for TB PCR, blood culture for TB (if septic)
[7]Suspect blood culture-negative endocarditis: Q fever, etc.
[8]Suspect hematologic disorders: peripheral blood smears, BM study, urine and serum protein electrophoresis, A/G, HTLV I+II antibody, consult hematologist
[9]Suspect malignancy: check tumor markers (AFP, CEA, CA-125, CA-199, PSA, SCC)
Potentially diagnostic clues – targeted examination
[10]Specific image study
[11]Biopsy of lymph node, liver, lung…..
[12]± surgery/ laparoscopy
No potentially diagnostic clues
Abdominal echo
Bone marrow study (biopsy and culture)
CT ± contrast of chest, abdomen, pelvis
± cardiac echo or TEE
± [67]Gallium scan
± FDG PET scan

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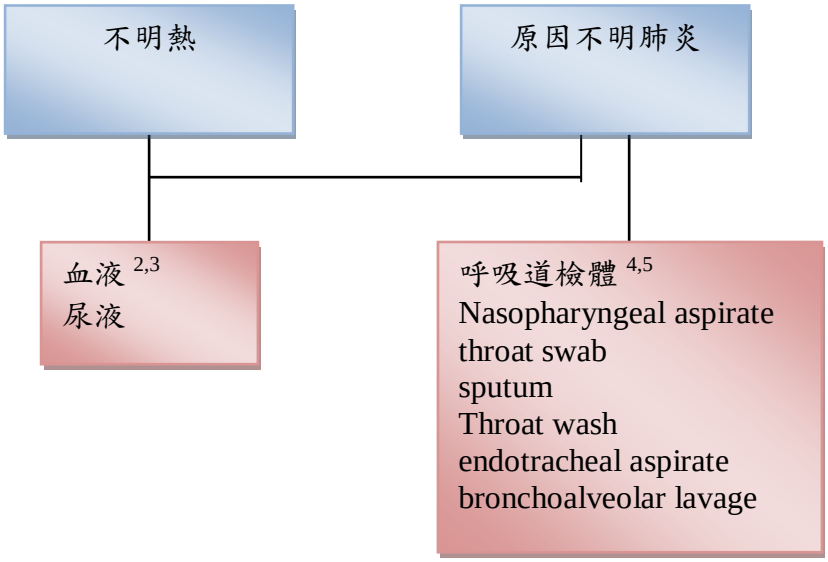
3) Harrison's principles of internal medicine. 16th edition
4) Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 7th edition, 2010.

圖四、臨床檢體收集流程 [1]

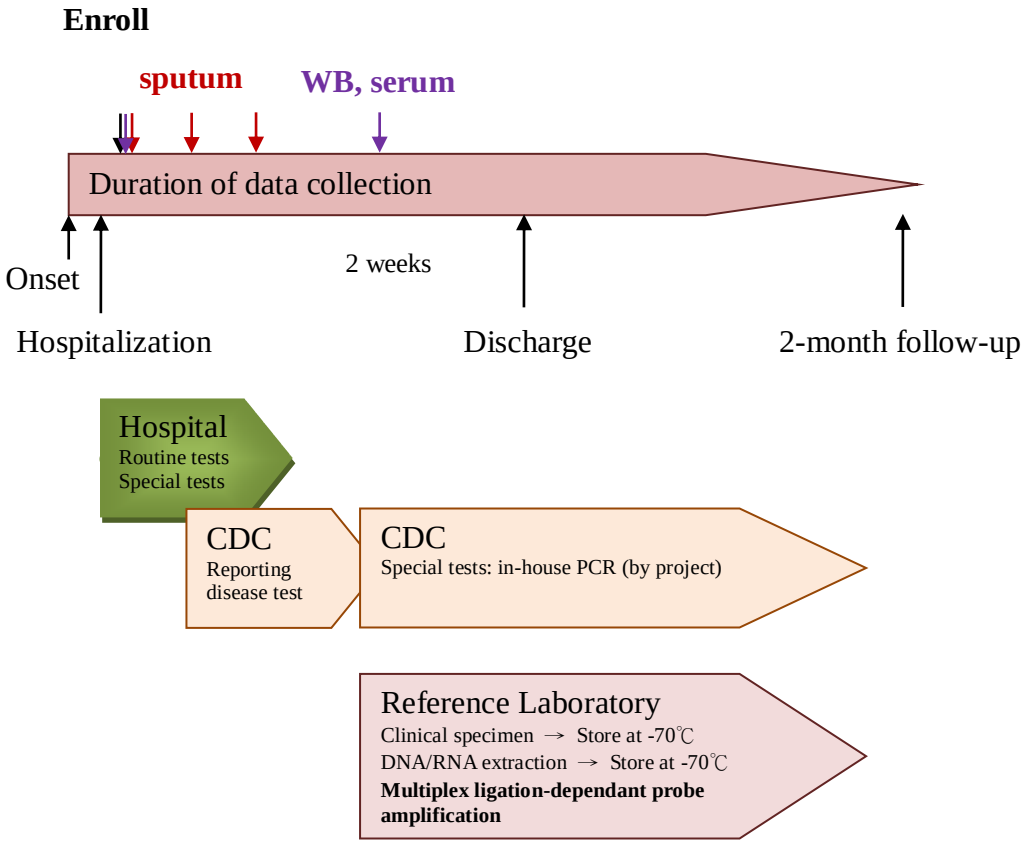


說明：

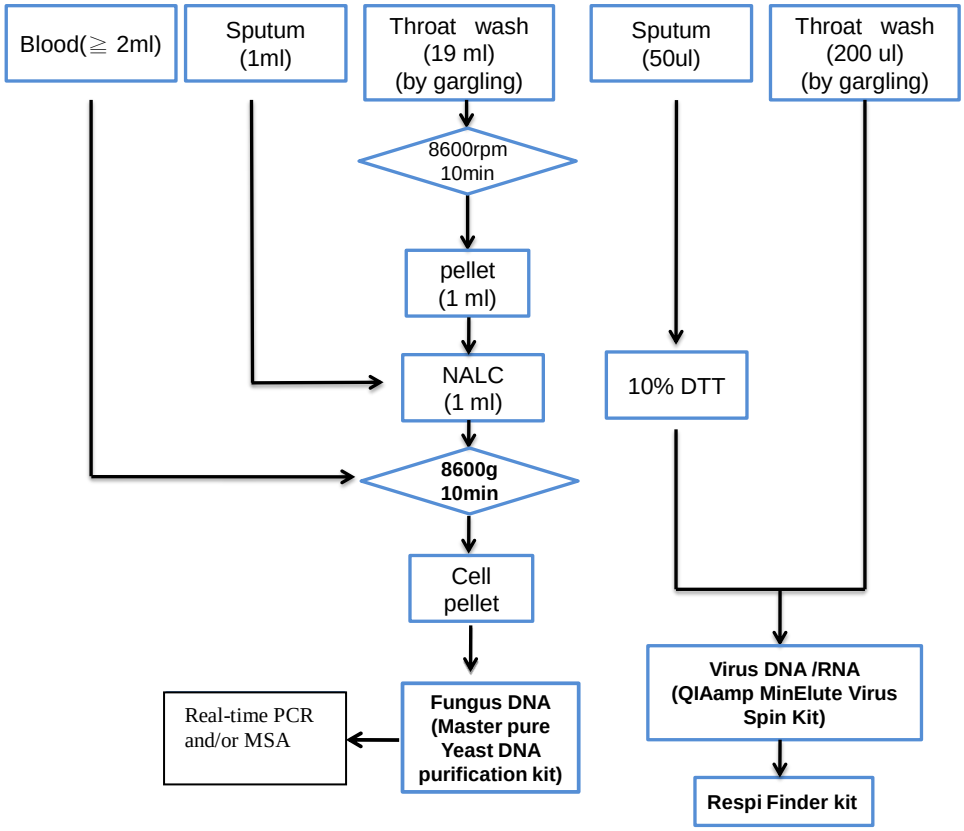
1. 臨床檢體收集種類及頻率：原則上依醫療常規，參**表三**。
2. 收案(或通報)(day 1)的前兩週：每週 2 次，共 4-5 次 (day 1, day 4±1, day 8±1, day 11±1, day 14 ±1)。Less than 5 specimens were collected because patients were discharged or fatal.
3. 本院的第一個血液檢體(CBC 管、血清生化管)收案(或通報)前的檢體，至 3 樓檢醫部收集(先查檢體號碼及送驗單位)
4. 呼吸道檢體**種類**因**病情**或**病原菌**特性而異，譬如
   (1) 沒有呼吸道分泌物者：取 throat swab 或 nasal swab 或 nasopharyngeal swab, gargling (throat wash)
   (2) nasopharyngeal aspirate: for all cases of influenza
   (3) 重症插管者：取 endotracheal aspirate,

   依病原菌特性，不同呼吸道檢體的檢出率可能不同，譬如
   (1) 流感病毒輕症病人快速抗原檢測採鼻咽拭子，檢出率比鼻腔拭子或咽喉拭子高。
   (2) 流感病毒重症病人可能 endo tracheal aspirate 檢出率比較高。better yield rate for severe cases of pandemic H1N1 influenza (personal communication)
5. 呼吸道檢體**頻率**因病情而異，譬如
   (1) All cases: 收案(通報)時
   (2) 肺囊胞肺炎: 收案(通報)時，3~4 天，及一週，共 3 次, since Oct 2010
6. 尿液檢體於收案(通報)時及一週後各收集一次 (尚未執行)
   (1) rapid antigen test (2) DNA extraction
7. 依醫療常規，照會胸腔科醫師以進行支氣管鏡檢查

圖五、研究設計


Enroll
sputum
WB, serum
Duration of data collection
Onset
Hospitalization
2 weeks
Discharge
2-month follow-up
Hospital
Routine tests
Special tests
CDC
Reporting disease test
CDC
Special tests: in-house PCR (by project)
Reference Laboratory
Clinical specimen → Store at -70℃
DNA/RNA extraction → Store at -70℃
Multiplex ligation-dependant probe amplification

圖六、檢體處理流程及分子診斷平台

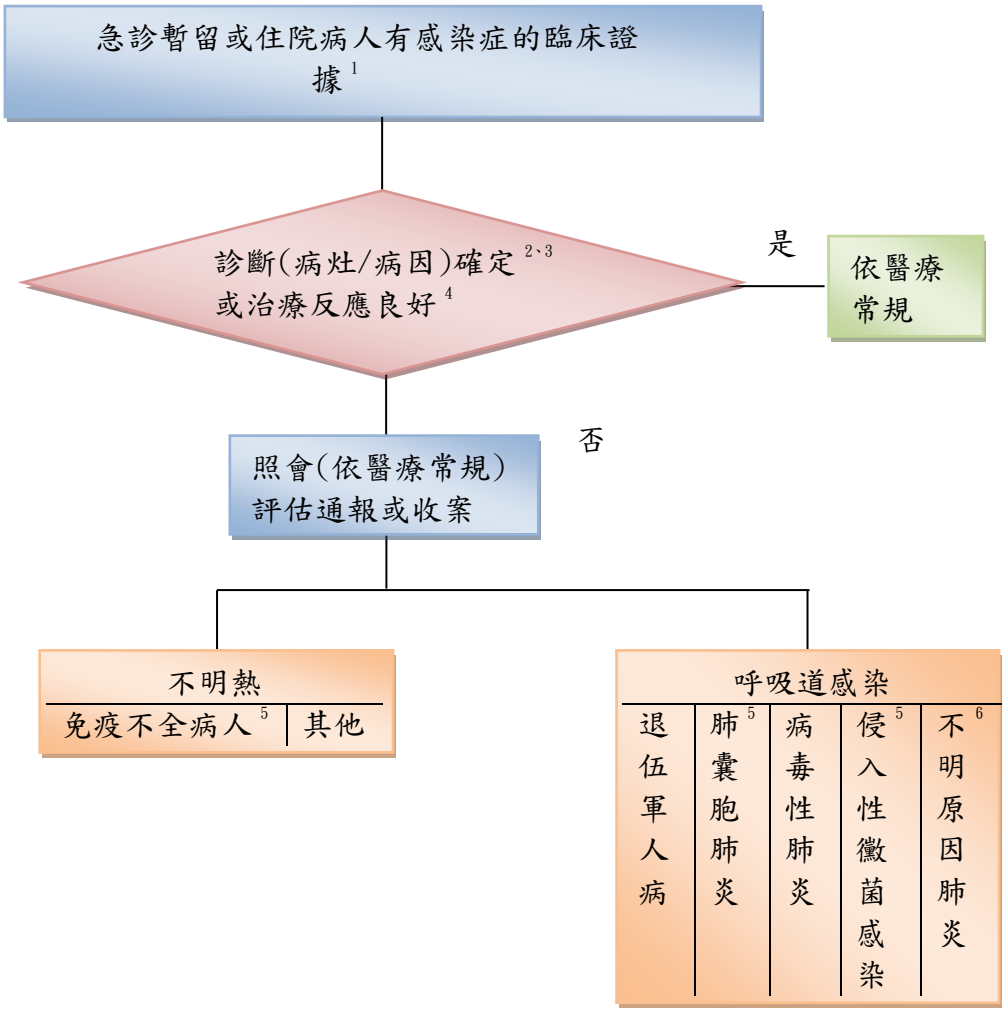


Nucleic acid extraction

Simulataneous purification of DNA and RNA was performed with the QIAamp MinElute Virus Spin Kit (QIAGEN). The initial samples was 200 ul of the respiratory specimens either eithiotreitol (DTT)-pretreated sputum or throat wash collected by gargling. The procedure is designed to ensure that there is no sample-to-sample cross-contamination and allows safe handeling of potentially infectious samples. Internal controls were introduced into the purification procedure and amplification procedures. Nucleic acid extracts were eluted in 30 ul eluton buffer and stored at -20C before molecular testing.

The procedure is highly suited for simultaneous processing of multiple smaples, yields pure nucleic acid in less than 1 hour. The eluent contains considerably more carrier RNA in the sample than viral nucleic acid (normally below 1 ug). The carrier RNA was added to enhance binding of viral nucleic acids to QIAamp membrane, especially if there are vey few target molecules in the sample and reduces the chance of viral RNA degradation. If cryoprecipitates of stored body fluids are visible, they can be pelleted by centrifugation of 6800xg for 3 minutes. The cleared supernatant should be removed and processed immediately without disturbing the pellet. This step will not reduce viral titers.

圖七、



註：

1. 感染性相對於非感染性全身性發炎反應(systemic inflammatory response syndrome)不容易區分。
2. 常見病灶包括血流感染、泌尿道感染、呼吸道感染、肝膽道感染、其他腹腔內感染、皮膚軟組織感染、骨骼關節感染、中樞神經感染等。呼吸道感染乃常見感染，但病原菌不容易判定。
3. 特殊病原菌如黴菌的確診不易，需依宿主條件、臨床條件及微生物證據全盤考慮，而不單依微生物證據之存在與否。
4. 治療反應依宿主情形及病原菌而異。
5. 高危險病人。

附錄一、

Case Report Form

[1]**Background:** Study sites: ☐ NTUH ☐ FEMH ☐ CSMUH ☐ E-DA ☐ KMUCMH
Name of persons completing form _____
Date of form completion (yyyy/mm/dd): _____

[2]**Demographics**
Name_____chart number: _____; [2.1]Gender: ☐ male ☐ female;
[2.2]Date of birth: _____
[2.3]The first date of this hospital visit: _____
[2.4]Admission day _____
([2.4.1] 來源: ☐ ER, ☐ OPD, ☐ Refer from other hospitals _____)
[2.5] 通報日 _____ [2.6]Discharge day _____
Information source: ☐ patient ☐ family members _____ ☐ other_____

[3]**Host factors** (通報日之前)
☐ [3.1]HIV-positive patients:
The latest CD4 count _____ cells/μL (date: _____)
The latest plasma HIV RNA load_____ copies/μL (date: _____)
☐ HAART use: regimen _____
☐ [3.2]Hematological malignancy _____, status_____
☐ [3.3]Solid organ tumor: type _____, status_____
☐ [3.4]Hematopoietic stem cell transplant: ☐ auto ☐ allo ☐ MUD ☐ PBSCT ☐ other_____
☐ [3.5]Solid organ transplant: ☐ kidney ☐ heart ☐ liver ☐ lung ☐ other _____
☐ [3.6]Recent myelosuppression therapy (<90 days): regimen _____
☐ [3.7]Recent immunosuppressants (<90 days) (average dose/day)
☐ prednisone (_____), ☐ azathioprine (_____),
☐ cyclosporine (_____),☐ cyclophosphamide (_____)
☐ tacrolimus (_____), ☐ sirolimus (_____),
☐ Other _____(_____)
☐ [3.8]Neutropenia (absolute neutrophil count <500 cells/mL),
ANC _____cells/mL, duration _____ days
☐ [3.9]iron overload, ferritin _____
☐ [3.10]Use of antimicrobial prophylaxis (<90 days), antibiotics: _____
☐ [3.11]Use of monoclonal antibody-based therapy:
☐ alemtuzumab (Campath) ☐ rituximab (Rituxan)
☐ infliximab(Remicade) ☐ etanercept (Enbrel) ☐ adalimumab
☐ interleukin-1 receptor antagonist: anakinra
☐ [3.12]Autoimmune disease: ☐ SLE ☐others _____
☐ [3.13]Diabetes mellitus: ☐ OHA; ☐ insulin; ☐ none;
☐ [3.14]HBV ☐ [3.15]HCV
☐ [3.16]Liver cirrhosis: ☐ Child A; ☐ Child B; ☐ Child C;
☐ [3.17]Chronic kidney disease (baseline serum creatinine: _____ mg/dL)
☐ [3.18]End stage renal disease: ☐ HD; ☐ PD;
☐ [3.19]Cardiovascular disease: ☐ Congestive heart failure; ☐ others_____
☐ [3.20]Chronic lung disease: ☐ COPD; ☐ asthma; ☐ others_____
☐ [3.21]Cerebral vascular disease: ☐ dementia ☐others_____

☐ [3.22]Implants: ☐ prosthetic valves (☐ mechanical ☐ xenograft)
☐ pacemaker ☐ carotid or coronary stents
☐ hip prosthesis ☐ knee prosthesis ☐ internal fixation
☐ Other underlying diseases _____

[4]Epidemiology
☐ [4.1]Community onset
☐ No recent hospitalization (<90 days)
☐ Recent hospitalization (<90 days)
☐ Reasons for hospitalization: ☐ infection ☐ non-infection ☐ unknown
☐ Antibiotic use: ☐ Yes ☐ No ☐ unknown
☐ [4.2]Nursing home residence ☐ [4.3]Respiratory care center residence
☐ [4.4]Healthcare-associated
☐ [4.5]Travel history (<3 months)
☐ Domestic (location _____; duration: _____)
☐ International (location _____; duration: _____)
☐ [4.6]Clusters (affected people: _____)
☐ [4.7]Animal contact history
☐ Rodents ☐ dogs ☐ cats ☐ pigs ☐ goats ☐ cattle ☐ sheep ☐ their secretions
☐ [4.8]Family history of infectious diseases (relationship: _____)
☐ Tuberculosis ☐ influenza ☐ other _____
Occupation: _____
☐ [4.9]Vaccination: ☐ pneumococcal (date:_____); ☐ influenza (date:_____);

[5]Clinical presentations
[5.1]Date of estimated onset of the disease: _____
[根據 ☐ Fever 或 ☐ cough 或 ☐ dyspnea) 判定發病日]
[5.2]Date of initial healthcare visit: _____ ☐ Outpatient ☐ Emergency room
[5.3]Fever: ☐ Yes (temperature: _____) ☐ No
[5.4]Consciousness: ☐ alert ☐ lethargic ☐ stuporous ☐ coma ☐ other:_____
[5.5]Constitutional symptoms: ☐ weight loss (____kg/___) ☐night sweats
☐ fatigue/malaise ☐ other: _____
[5.6]HEENT: ☐ rhinorrhea ☐ epistaxis ☐ sinus pain ☐ ear pain ☐ sore throat ☐ odynophagia
[5.7]☐ lymphoadenopathy (location: _____; size _____)
☐ necrotic lesion (location: _____) ☐ other: _____
[5.8]Respiratory tract: ☐ cough ☐ wheeze ☐ haemoptysis ☐ other:_____
[5.9]Cardiovascular system: ☐ chest pain ☐ shortness of breath ☐ other: _____
[5.10]Gastrointestinal system: ☐ nausea ☐ vomit ☐ diarrhea (frequency: ___/day
☐ watery ☐ bloody ☐ loose stool) ☐ abdominal pain location:_____)
☐ other: _____
[5.11]Urinary tract: ☐ incontinence ☐ dysuria ☐ frequency ☐ urgency
☐ haematuria ☐ other: _____
[5.12]Skin lesions (location: _____): ☐ rash ☐ petechia ☐ ecchimosis
☐ eschar ☐ other _____)
[5.13]Neurologic system: ☐ headache ☐ vertigo ☐ unstable gait ☐ photophobia
☐ phonophobia(怕吵)
Other symptoms/findings: _____

[6]Clinical diagnosis (not include underlying diseases)

[6.1]at admission: _____

[6.2]at discharge: _____

Category

☐ [6.3]Fever of unknown origin (FUO): ☐ Fever >38.3 C, **AND** ☐ Fever for more than 3 weeks, **AND** ☐ Fever >2 outpatient visits or 3 days in hospitalization)

☐ Classic FUO ☐ Nosocomial FUO ☐ Neutropenic FUO ☐ HIV-associated FUO

☐ [6.4]Pneumonia:

☐ Presence of new infiltrates on a chest radiograph, **AND**

☐ Clinical signs of acute lower respiratory tract infection:

☐cough ☐fever (>38.3C) or chills or hypothermia (<35.5C)

☐ unexplained hypotension

☐ abnormal white blood cell count (>11x10^9/L or <3x10^9/L)]

[7]Microbiology

☐ Routine

Sputum smear Date: _____ (請根據 ☐ 通報日之前或後 3 天，或 ☐ 發燒或呼吸道症狀開始前或後 3 天之第一個檢驗結果)

PMN ☐>25 ☐<25; Epithelium ☐>10 ☐<10;

☐ no bacteria visible; ☐ bacteria visible_____

Sputum culture (請根據 ☐ 通報日之前或後 3 天，或 ☐ 發燒或呼吸道症狀開始前或後 3 天之第一個檢驗結果)

Date: _____, isolate: _____

☐[7.1]Urinary pneumococcal antigen (date:_____): ☐Positive ☐Negative

☐[7.2]Sputum *Clamydia* antigen (or PCR) (date: _____): ☐Positive ☐Negative

☐[7.3]*Mycoplasma* antibody 1st IgM (date: _____): ☐Positive (titer _____) ☐Negative

☐[7.4]*Mycoplasma* antibody 1st IgG (date: _____): ☐Positive (titer _____) ☐Negative

☐[7.5]*Mycoplasma* antibody 2nd IgM (date: _____): ☐Positive (titer _____) ☐Negative

☐[7.6]*Mycoplasma* antibody 2nd IgG (date: _____):☐Positive (titer _____) ☐Negative

☐[7.7]Urinary *Legionella* antigen (date:_____): ☐Positive ☐Negative

☐[7.8] Serum Cryptococcal Ag (date: _____): ☐Pos (titer_____) ☐Negative

☐[7.9] Cryptococcal Ag (☐CSF ☐other _____) (date: ____): ☐Pos (titer____) ☐Negative

☐[7.10] *Aspergillus* Ag (☐serum ☐BAL ☐other _____)

(date: _____): ☐Pos (value_____) ☐Negative

[8]Routine laboratory findings: (根據 ☐ 通報日之前或後 3 天；或 ☐ 發燒或呼吸道症狀開始之前或後 3 天內之第一個檢驗結果)

WBC _____, Seg _____%, Lym_____%, aty lym _____%, Hb _ ___g/dL,

Plt_____k/uL, CRP _____. Procalcitonin _____ LDH_____CPK _____;

GOT_____ GPT_____ ALP_____ GGT_____ BUN _____ Cr_____

[9]Image study (請以 chest CT 為主，若沒有 chest CT 則根據 ☐ 通報日之前或後 3 天 或 ☐ 發燒或呼吸道症狀開始之前或後 3 天內之第一張 CXR)

Date: _____, radiology finding by ☐ chest X-ray or ☐ chest CT

☐ nodules (☐single ☐multiple) ☐ halo sign ☐mass

☐ consolidation ☐ patches ☐ cavitary ☐ air-cresent sign

☐ infiltrations ☐ interstitial ☐ ground glass

☐ septic lesions ☐ pleural effusion (☐ right ☐ left ☐ bilateral)

☐ other finding _____

[10]Pathology

Date: _____; site: _____

[10.1]diagnosis: _____

Date: _____; site: _____;

[10.2]diagnosis: _____

Date: _____; site: _____;

[10.3]diagnosis: _____

☐ **[11]Complications during hospitalization**

☐ [11.1]Respiratory failure requiring mechanical ventilation

☐ [11.2]Respiratory failure requiring additional ECMO support

☐ [11.3]Acute renal failure requiring : ☐ CVVH, ☐ CAVH

[12]Outcome

☐ [12.1]In-hospital mortality (date of death: _____)

☐ [1]Death caused by infectious disease: _____;

☐ [2]Death caused by underlying diseases:_____;

☐ [3]Others:_____;

☐ [12.2]Morbidity

☐ [1]Tracheostomy; ☐ [2]ventilator dependent; ☐ [3]Dialysis;

☐ [4]Others _____

[13]CDC Laboratory findings

☐ PJP (date: _____): ☐Positive (Specimen_____); ☐Negative

☐ *Legionella* (date: _____): ☐Positive (Specimen_____); ☐Negative

☐ Leptospirosis (date: _____): ☐Positive (Specimen_____); ☐Negative

☐ Q fever (date: _____): ☐Positive (Specimen_____); ☐Negative

☐ Scrub typhus (date: _____):☐Positive (Specimen_____);☐Negative

☐ Endemic typhus (date: _____): ☐Positive (Specimen_____); ☐Negative

☐ Others_____ (date: _____): ☐Positive (Specimen_____);☐Negative

☐ [14]**Specimens for respiratory viral panel** (date: _____)

☐ Influenza A (date: _____): ☐Positive ☐Negative

☐ Influenza B (date: _____): ☐Positive ☐Negative

☐ Respiratory syncytial virus (date: _____): ☐Positive ☐Negative

☐ Parainfluenza virus (date: _____): ☐Positive ☐Negative

☐ Adenovirus (date: _____): ☐Positive ☐Negative

☐ Human metapneumovirus (date: _____): ☐Positive ☐Negative

☐ Others_____ (date: _____): ☐Positive ☐Negative

名稱	通報定義： 符合「臨床病例」或「實驗室診斷」者，即可進行通報
退伍軍人病 (Legionellosis)	(一)臨床病例：符合臨床症狀：包括倦怠感、畏寒、肌肉酸痛、頭痛、發燒、頭昏、咳嗽、噁心、腹痛等身體不適、並以肺炎為主要症狀，及可能併有腦病症、下痢及其他器官受波及或多器官受侵犯等症狀者。 (二)實驗室診斷： 1.由肺組織、呼吸道分泌物、胸膜液、血液或其他正常無菌的部位，分離退伍軍人桿菌(Legionella)。 2.以直接免疫螢光抗體試驗在肺組織、呼吸道分泌物或胸膜液檢驗嗜肺性退伍軍人桿菌(L. pneumophila)。 3.以間接免疫螢光抗體試驗檢測血清抗體效價，比較恢復期(4～12 週)抗體效價是否比發病初期效價有 4 倍以上增加，且≧128。 4.以酵素連結免疫分析法或快速免疫呈色膜法檢驗尿中嗜肺性退伍軍人桿菌血清型第一型(L. pneumophila serogroup I)之抗原。
鉤端螺旋體病 (Leptospirosis)	需以下兩者兼備： 1.發病前 1 個月內曾有接觸動物、野外活動或暴露於被感染動物尿液污染之環境(如污水、溼土等) 2.出現急性發燒、頭痛、肌肉痛(尤其常見小腿肚痛)、腹痛、腹瀉、倦怠，尤其伴有下列任一種 臨床表現者： (1)結膜充血(conjunctival suffusion) (2)腦膜炎症狀(meningeal irritation)及無菌性腦膜炎(aseptic meningitis) (3)無尿、少尿或蛋白尿(anuria、oliguria or proteinuria) (4)黃疸(jaundice) (5)急性腎功能不全(acute renal insuffi ciency) (6)出血傾向(腸道或肺部)(gastro-intestinal orlung hemorrhage)
流感重症	出現類流感症狀*後四週內，發生符合以下臨床狀況至少一項者，即可進行通報： (一)肺部併發症(Pulmonary complications)且住院者 (二)神經系統併發症(Neurological complications) (三)心肌炎(myocarditis)或心包膜炎(pericarditis) (四)侵襲性細菌感染(Invasive bacterial infection) (五)其他(Others)：非符合上述四項臨床症狀，但個案需於加護病房治療，或死亡者。 (*類流感症狀：需同時符合「突然發病、有發燒(耳溫≧38℃)及呼吸道症狀」、「肌肉酸痛或頭痛或極度倦怠感」、「需排除單純性流鼻水、扁桃腺炎及支氣管炎」等三項條件者。)
不明原因肺炎 *Pneumocystis jiroveci* pneumonia (PJP)	

附錄三、肺炎及病因定義

Case definition

Patients who were > 16 year old with a diagnosis of “lower respiratory infection” were examined for the possibility of community-acquired pneumonia. Patients who were enrolled must fulfill the following two criteria: 1) a chest radiograph (CXR) taken within 48 h after initial visit revealing a new infiltrate consistent with pneumonia; and 2) presenting with two of the following clinical criteria: productive cough, fever (> 38.3 ^{O}C) or chills or hypothermia (< 35.5 ^{O}C), unexplained hypotension, or abnormal white blood cell count (> 11x10^{9}/L, or < 3x10^{9}/L).

Assignment of etiology

Establishment of pneumonia with specific etiologic agents includes definite and probable categories following published criteria and guidelines [9-11]. A definite agent is assigned when 1) the isolate was cultured from blood or pleural fluid; 2) ≥ 4 fold rise in IgG antibody titer to *L. pneumophila* (to ≥ 1:128), *C. pneumoniae* (to ≥ 1:256), or to any of the respiratory virus antigens tested, or a seroconversion of antibodies to *M. pneumoniae* based on manufacturer’s criteria; 3) detection of *L. pneumophila* antigen in urine, or 4) detection of *S. pneumoniae* antigen in urine plus isolation of *S. pneumoniae* from purulent sputum. A probable agent is assigned when 1) a pathogen was isolated as a predominant organism from a purulent sputum in which a compatible organism was seen intracellularly, as a predominant organism, or in at least moderate amount on Gram stain; or 2) detection of *S. pneumoniae* antigen in urine.

附錄四、2009 肺炎及不明熱相關法定傳染病之通報及確診個案數統計表

	A. 台大醫院			B. 亞東紀念醫院			C.中山醫學大學附設醫院			D.高雄醫學大學附設中和紀念醫院			E.財團法人義大醫院			總計		
法傳疾病名稱	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比
肺炎或不明熱																		
結核病(Tuberculosis)	496	392	79%	23	16	70%	131	127	97%	353	332	94%	458	420	92%	**1461**	**1287**	**88%**
肺炎																		
退伍軍人病(Legionellosis)	32	9	28%	9	1	11%	0	0	0%	23	0	0%	5	3	60%	**69**	**13**	**18.8%**
鉤端螺旋體病(Leptospirosis)	58	1	2%	24	0	0%	23	0	0%	37	10	27%	44	10	23%	**186**	**21**	**11%**
類鼻疽(Melioidosis)	2	0	0%	0	0	0%	0	0	0%	3	3	100%	7	7	100%	**12**	**10**	**83%**
流感重症	243	44	18%	80	21	26%	40	15	38%	24	14	58%	35	9	26%	**422**	**103**	**24%**
不明原因肺炎(virus)	0	0	0%	0	0	0%	0	0	0%	0	0	0%	0	0	0%	**0**	**0**	**0%**
Pneumocystis jiroveci pneumonia	47	31	66%	0	0	0%	0	0	0%	0	0	0%	0	0	0%	**47**	**31**	**66%**
不明熱																		
恙蟲病 (Scrub typhus)	64	8	13%	23	1	4%	28	0	0%	127	8	6%	123	10	8%	**365**	**27**	**7%**
地方性斑疹傷寒 (Endemic typhus)	12	2	17%	9	1	11%	6	0	0%	118	0	0%	120	2	2%	**265**	**5**	**2%**

流行性斑疹傷寒 (Epidemic typhus)	0	0	0%	0	0	0%	0	0	0%	0	0	0%	0	0	0%	**0**	**0**	**0%**
Q 熱 (Q fever)	40	0	0%	14	0	0%	24	0	0%	129	2	2%	121	11	9%	**328**	**13**	**4%**
鸚鵡熱	0	0	0%	0	0	0%	0	0	0%	0	0	0%	1	0	0%	**1**	**0**	**0%**
登革熱	27	9	33%	5	1	20%	3	0	0%	107	54	50%	27	5	19%	**169**	**69**	**41%**
登革出血熱/登革休克症候群	0	0	0%	5	1	20%	0	0	0%	2	2	100%	0	0	0%	**7**	**3**	**43%**
屈公病	1	0	0%	0	0	0%	0	0	0%	2	1	50%	0	0	0%	**3**	**1**	**33%**
瘧疾 (Malaria)	5	2	40%	0	0	0%	1	1	100%	1	0	0%	0	0	0%	**7**	**3**	**43%**
貓抓病 (Cat scratch)	12	0	0%	2	0	0%	5	0	0%	5	0	0%	2	0	0%	**26**	**0**	**0%**
總計	1039	498	48%	194	42	22%	261	143	55%	931	426	46%	943	477	51%	3368	1586	47%

2010 肺炎及不明熱相關法定傳染病之確診統計表

	A. 台大醫院			B. 亞東紀念醫院			C.中山醫學大學附設醫院			D.高雄醫學大學附設中和紀念醫院			E.財團法人義大醫院			總計		
法傳疾病名稱	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比	通報個案數	確診個案數	確診百分比
肺炎或不明熱																		
結核病(TB)	489	389	80%	330	313	95%	134	38	28%	385	314	82%	378	300	79%	1716	1354	79%
肺炎																		
退伍軍人病(Legionellosis)	35	4	11%	22	1	5%	2	2	100%	39	2	5%	8	3	38%	106	12	11%
鉤端螺旋體病(Leptospirosis)	104	4	4%	28	2	7%	23	0	0%	32	0	0%	41	3	7%	228	9	4%
類鼻疽(Melioidosis)	0	0	0%	0	0	0%	0	0	0%	1	1	100%	5	5	100%	6	6	100%
流感重症	87	20	23%	50	11	22%	7	3	43%	46	18	39%	51	33	65%	241	85	35%
不明原因肺炎(virus)	9	4	44%	21	10	48%	0	0	0%	0	0	0%	0	0	0%	30	14	46%
Pneumocystis jiroveci pneumonia (PCP or PJP)	155	83	54%	5	0	0%	0	0	0%	6	0	0%	2	1	50%	168	84	50%
不明熱																		

恙蟲病(Scrub typhus)	110	6	5%	28	0	0%	28	0	0%	114	8	7%	99	4	4%	379	18	5%
地方性斑疹傷寒(Endemic typhus)	63	2	3%	17	1	6%	3	0	0%	105	1	1%	99	4	4%	287	8	3%
流行性斑疹傷寒(Epidemic typhus)	4	0	0%	0	0	0%	0	0	0%	0	0	0%	0	0	0%	4	0	0%
Q熱(Q fever)	69	0	0%	38	2	5%	25	0	0%	115	3	3%	101	8	8%	348	13	4%
鸚鵡熱	0	0	0%	0	0	0%	1	0	0%	1	0	0%	0	0	0%	2	0	0%
登革熱	33	11	33%	11	4	36%	6	0	0%	333	167	50%	42	11	26%	425	193	45%
登革出血熱/登革休克症候群	0	0	0%	0	0	0%	0	0	0%	4	4	100%	0	0	0%	4	4	100%
屈公病	2	0	0%	0	0	0%	1	0	0%	4	0	0%	1	0	0%	8	0	0%
瘧疾 (Malaria)	8	1	13%	1	1	100%	0	0	0%	0	0	0%	0	0	0%	9	2	22%
貓抓病 (Cat scratch)	13	3	23%	6	1	17%	2	1	50%	14	3	21%	2	0	0%	37	8	22%

附錄五

退伍軍人症病例收案成果分析

A. 疑似退伍軍人症病例回朔(Retrospective)分析研究

為深入了解在台灣引起一般住院病人**非典型肺炎病原菌**，所以針對台灣北中南五大醫學中心，包括台大醫院、亞東紀念醫院、台中中山醫院、義大醫院、高雄醫學大學附設中和紀念醫院；從通報台灣疾病管制局退伍軍人症疑似病例中做病例回朔(Retrospective)分析研究。個案收集是從 2009/1/1 至 2009/12/31 為止。病人收案定義需要同時符合以下三個條件- 1)病人年齡大於或等於 16 歲；2)因為肺炎而住院病人；3)符合通報台灣疾病管制局疑似或確定退伍軍人症病例病人。

病人資料收集是藉由 case report form (CRF)來完成。CRF 資料包括 1)病人流行病學(age, gender, underlying diseases, recent hospitalization (<90 days), residents of long-term care facility....)；2)病人求診、住院及通報台灣疾病管制局日期；3)住院可能診斷、併發症與否、預後、微生物培養結果等等。同時台灣疾病管制局劉博士實驗室將通報疑似退伍軍人症病人痰檢體針對病毒(influenza A H1，H3，or pH1N1；influenza B；RSV-AB；adenovirus；human metapneumovirus； rhinovirus； HSV1； HSV2； CMV； parainfluenza type 1，type 2，or type 3； enterovirus)進一步做 RT-PCR。

因為台中中山醫院在研究期間，無任何通報疑似退伍軍人症病例。所以最後是從四大醫學中心，包括台大醫院、亞東紀念醫院、義大醫院、高雄醫學大學附設中和紀念醫院；總共收集了 69 個退伍軍人症疑似病例。不過有 2 個病例是小於 16 歲，所以最後以 67 個退伍軍人症疑似病例做病例回朔分析 (Figure 1)。其中確診個案數是 13 個，全部退伍軍人症確診百分比是 19.4%。進一步分析，台大醫院有 30 個通報個案數，確診個案數是 9 個，確診百分比是 30.0%；亞東紀念醫院有 9 個通報個案數，確診個案數是 1 個，確診百分比是 11.1%；義大醫院有 5 個通報個案數，確診個案數是 3 個，確診百分比是 60.0%及高雄醫學大學附設中和紀念醫院有 23 個通報個案數，確診個案數是 0 個，確診百分比是 0% (Table 1)。經過一連串病例檢體檢查、分析，及痰檢体 RT-PCR for 13 virus panel(因為有些檢體不夠，所以只有 49 痰檢体進行 RT-PCR)。最後，有找到可能致病菌病例數是 17 個(25.3%) (Table 1)。但仍然有將近 55.2%病人(37 個病例數)找不到可能致病菌(Table 1). 進一步分析、比較這 3 組病人 (包括退伍軍人症確診病例、有找到可能致病

菌病例及找不到可能致病菌病例)流行病學之相關特性發現: 1) 有找到可能致病菌這一組病人較年輕 (mean age 54.2) 且男性比例最低 (58.8%); 2) 呼吸衰竭需要插管比例最高 (76.5%); 3) 住院天數較久 (median 28 days) (Table 2). 但以退伍軍人症確診這一組病人男性比例最高 (92.3%)且死亡率最高 (38.5%) (Table 2)。且從我們這個病例回朔分析研究發現，這些找到可能致病菌，有將進一半都是藉由痰檢体 RT-PCR for 13 virus panel 找到。可能致病菌包括 influenza A (H3N2, pH1N1), adenovirus, human metapneumovirus, and CMV。至於這些沒有找到可能致病菌病例，其檢體需更進一步分析。

B. 疑似退伍軍人症病例前瞻性收案分析研究

病例個案收集是從 2009/01/01 至 2011/6/30 為止，總共收集了 186 個通報台灣疾病管制局退伍軍人症疑似病例。其中確診個案數是 24 個，全部確診百分比是 12.9%。依通報醫院分析，台大醫院有 87 個通報個案數，確診個案數是 18 個，確診百分比是 20.7%；亞東紀念醫院有 23 個通報個案數，確診個案數是 3 個，確診百分比是 13.0%；及高雄醫學大學附設中和紀念醫院有 76 個通報個案數，確診個案數是 3 個，確診百分比是 3.9%。

進一步，將收案 186 個通報台灣疾病管制局退伍軍人症疑似病例作回朔(Retrospective)分析研究。因為有 4 個個案是小於 16 歲，且有些個案 CRF 填寫不完全；所以最後是分析 173 個病例。包括台大醫院 82 個個案、亞東紀念醫院 23 個個案及高雄醫學大學附設中和紀念醫院 68 個個案。確診退伍軍人症個案數是 21 個，確診百分比是 12.1% (21/173)。最後，有找到可能致病菌病例數是 39 個(22.5%) (Figure 2)。但有將近 65.3%病人(113 個病例數)找不到可能致病菌(Figure 2)。這收案 173 個病例的流行病學詳細資料請見於 Table 3. 收案病例男性佔 69.4%，平均年齡是 59.8±17.3 歲。整體來說，有將近 68.2%收案病例有 underlying disease。最常見 underlying diseases 是心血管疾病。平均將近 55.5%收案病例接受插管治療，致死率將近 24.3%。北部和南部收案病例流行病學特性相差不大。Table 4 為收案 173 病例引起肺炎的可能致病菌。除了退伍軍人症外，引起非典型肺炎致病菌在台灣是肺結核、influenz A、 Mycoplasma、 Chalamydia、 endemic typhus、scrub typhus、 Pneumocystic carinii、 CMV 等等。

依照通報退伍軍人症程序後，台灣疾病管制局會保存通報病人痰檢體；同時我們又

會額外收集病人檢體，包括全血、血清及其他呼吸道檢體。但因為檢體實在不易收集，所以總共只有 24 個退伍軍人症疑似個案有額外收集到臨床檢體，檢體總數共 85 個。其中包括全血(whole blood) 40 個，血清(serum) 41 個，呼吸道檢體 2 個，及腦脊髓液(CSF)檢體 2 個。

進一步，將呼吸道檢體抽取 DNA 及 RNA，以 RespiFinder pathogen identification panel 針對 19 種 pathogens 監測分析。已完成檢驗的兩個檢體，其中一個檢體偵測到 influenza A；而另一個檢體則無偵測到任何 pathogen，此個案檢體待未來進一步做分析是否有 novel etiology。

Table 1. The possible etiology of 67 hospitalized patients with pneumonia from 4 medical centers in Taiwan, 2009/1/1- 2009/12/31

	NTUH N=30	FEMH N=9	KMUCMH N=23	E-DA N=5	Total N=67
No. of patients with confirmed legionellosis (%)	9 (30%)	1 (11.1%)	0	3 (60%)	13 (19.4%)
No. of patients with other etiology (%)	7 (23.3%)	5 (55.6%)	5 (21.7%)	0	17 (25.3%)
CA-MRSA	0	1	0	0	1
Enterobacter aerogenes	0	1	0	0	1
TB	**3**	0	0	0	**3**
Mycoplasma pneumoniae	0	2	0	0	2
Chlamydia	1	0	0	0	1
Influenza A/pH1N1	0	1	1	0	2
Influenza A/H3N2	1	0	0	0	1
Human metapneumovirus	0	0	1	0	1
Adenovirus	1	0	1	0	2
CMV	3	0	1	0	4
Scrub typhus	0	0	1	0	1
Endemic typhus	1	0	0	0	1
No. of patients without identified etiology (%)	14 (46.7%)	3 (33.3%)	18 (78.3%)	2 (40%)	37 (55.2%)

Table 2. Comparison of demographic features and other characteristics of 67 patients according to different etiologies of atypical pneumonia, 2009/1/1- 2009/12/31

Parameter	Patients with Legionellosis	Patients with possible etiology	Patients without identified etiology	Overall
No. of patients	13	17	37	67
Age, y				
Mean±SD	66.7±13.0	54.2±20	65.3±16.7	62.8±17.5
16 - 44 (%)	1 (7.7%)	6 (35.3%)	5 (13.5%)	12 (17.9%)
45 - 64 (%)	4 (30.8%)	5 (29.4%)	10 (27.0%)	19 (28.4%)
≧65 (%)	8 (61.5%)	6 (35.3%)	22 (59.5%)	36 (53.7%)
Male (%)	12 (92.3%)	10 (58.8%)	25 (67.6%)	47 (70.1%)
Previous admission (<90 days, %)	4 (30.8%)	4 (23.5%)	14 (37.8%)	22 (32.8%)
Underlying diseases (%)	11 (84.6%)	14 (82.4%)	31 (83.8%)	56 (83.6%)
HIV (%)	1 (7.7%)	0	0	1 (1.5%)
Malignancy				
Hematological (%)	1 (7.7%)	0	0	1 (1.5%)
Solid organ (%)	2 (15.4%)	2 (11.8%)	4 (10.8%)	8 (11.9%)
Transplantation				
Bone marrow stem cells (%)	0	0	0	4 (6.0%)
Solid organ (%)	2 (15.4%)	1 (5.9%)	1 (2.7%)	0
Autoimmune diseases (%)	1 (7.7%)	1 (5.9%)	3 (8.1%)	5 (7.5%)
Diabetes mellitus (%)	2 (15.4%)	3 (17.6%)	7 (18.9%)	12 (17.9%)
Chronic liver disease (%)	1 (7.7%)	1 (5.9%)	4 (10.8%)	6 (9.0%)
Chronic kidney diseases (%)	4 (30.8%)	4 (23.5%)	5 (13.5%)	13 (19.4%)
Cardiovascular diseases (%)	6 (46.2%)	8 (47.1%)	14 (37.8%)	28 (41.8%)
Chronic lung diseases (%)	4 (30.8%)	1 (5.9%)	5 (13.5%)	10 (14.9%)
Cerebral vascular diseases (%)	2 (15.4%)	2 (11.8%)	5 (13.5%)	9 (13.4%)
Respiratory failure with ventilation (%)	7 (53.8%)	13 (76.5%)	20 (54.1%)	40 (59.7%)
Respiratory failure with ECMO support (%)	1 (7.7%)	1 (5.9%)	1 (2.7%)	3 (4.5%)
Duration from 住院 to 通報 (days), median(IQR)	6.5 (5.3-7)	3 (2-5)	4 (2-6)	4 (3-7)
Length of stay (days), median (IQR)	16 (12-23.8)	28 (13-44)	19 (15-50)	19 (13-41.4)
Mortailty (%)	5 (38.5%)	5 (29.4%)	9 (24.3%)	19 (28.4%)

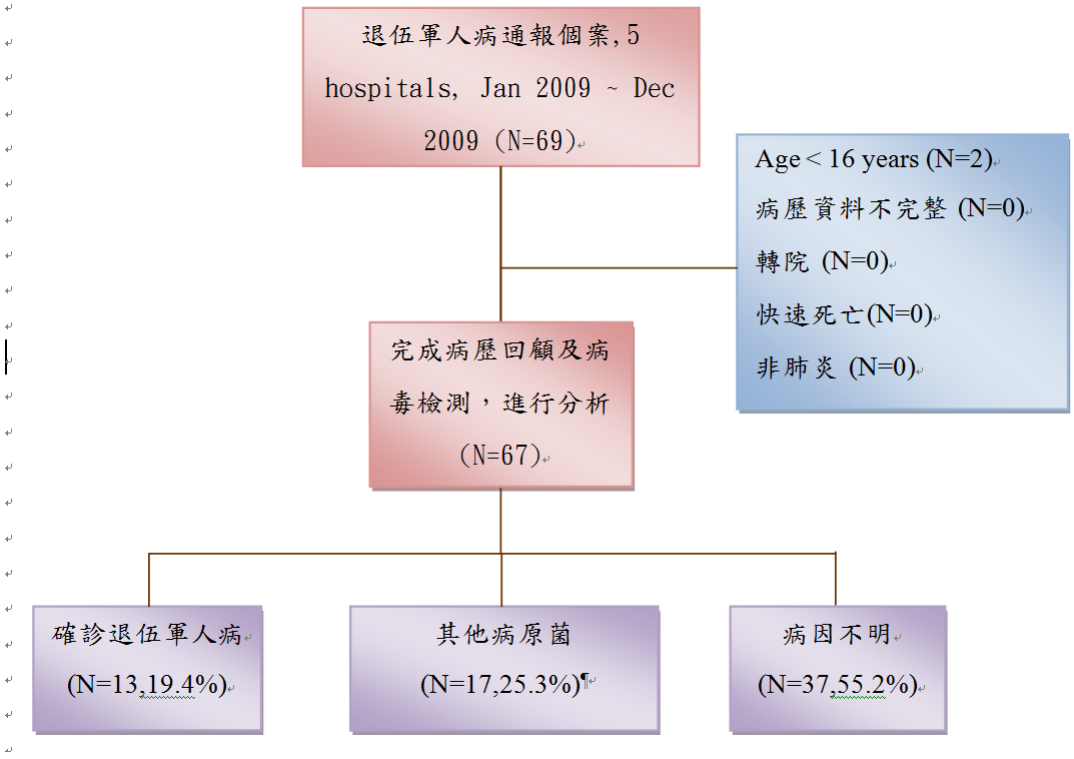
Table 3. The characteristics of 173 hospitalized patients with atypical pneumonia from Jan 1, 2009 through Jun 30, 2011

	NTUH	FEMH	KMUCMH	Overall
No. of patients	82	23	68	173
Age, y				
Mean±SD	61.2±17.2	61.3±16.1	56.9±17.9	59.8±17.3
16 - 44 (%)	13 (15.9%)	2 (8.7%)	13 (19.1%)	28 (16.1%)
45 - 64 (%)	34 (41.5%)	9 (39.1%)	19 (27.9%)	62 (35.8%)
≧65 (%)	35 (42.6%)	12 (52.2%)	36 (52.9%)	83 (48.0%)
Male (%)	59 (72.0%)	14 (60.9%)	47 (69.1%)	120 (69.4%)
Residents of long-term care facility (%)	2 (2.4%)	2 (8.7%)	10(14.7%)	14 (8.1%)
Previous admission (<90 days, %)	2 (2.4%)	3 (13.0%)	0	5 (2.9%)
Underlying diseases (%)	58 (70.7%)	21 (91.3%)	39 (57.4%)	118 (68.2%)
HIV (%)	3 (5.2%)	0	1 (2.6%)	4 (3.4%)
Malignancy				
Hematological (%)	3 (5.2%)	0	0	3 (2.5%)
Solid organ (%)	10 (17.2%)	3 (14.3%)	0	13 (11.05)
Transplantation				
Bone marrow stem cells (%)	1 (1.7%)	0	0	1 (0.8%)
Solid organ (%)	3 (5.2%)	0	1 (2.6%)	4 (3.4%)
Autoimmune diseases (%)	6 (10.3%)	4 (19.0%)	1 (2.6%)	11 (9.3%)
Diabetes mellitus (%)	19 (32.8%)	12 (57.1%)	7 (17.9%)	38 (32.2%)
Chronic liver disease (%)	11 (19.0%)	0	8 (20.5%)	19 (16.1%)
Chronic kidney diseases (%)	16 (27.6%)	4 (19.0%)	3 (7.7%)	23 (19.5%)
Cardiovascular diseases (%)	23 (39.7%)	15 (71.4%)	23 (59.0%)	61 (54.0%)
Chronic lung diseases (%)	10 (17.2%)	5 (23.8%)	5 (12.8%)	20 (17.7%)
Cerebral vascular diseases (%)	5 (8.6%)	4 (19.0%)	6 (15.4%)	15 (13.3%)
Respiratory failure with ventilation (%)	47 (57.3%)	21 (91.3%)	28 (41.2%)	96 (55.5%)
Respiratory failure with ECMO support (%)	7 (8.5%)	4 (17.4%)	1 (1.5%)	12 (6.9%)
Mortailty (%)	28 (34.1%)	4 (17.4%)	10 (14.7%)	42 (24.3%)

Table 4. The possible etiology of 173 hospitalized patients with pneumonia from 4 medical centers in Taiwan, 2009/1/1- 2011/06/30

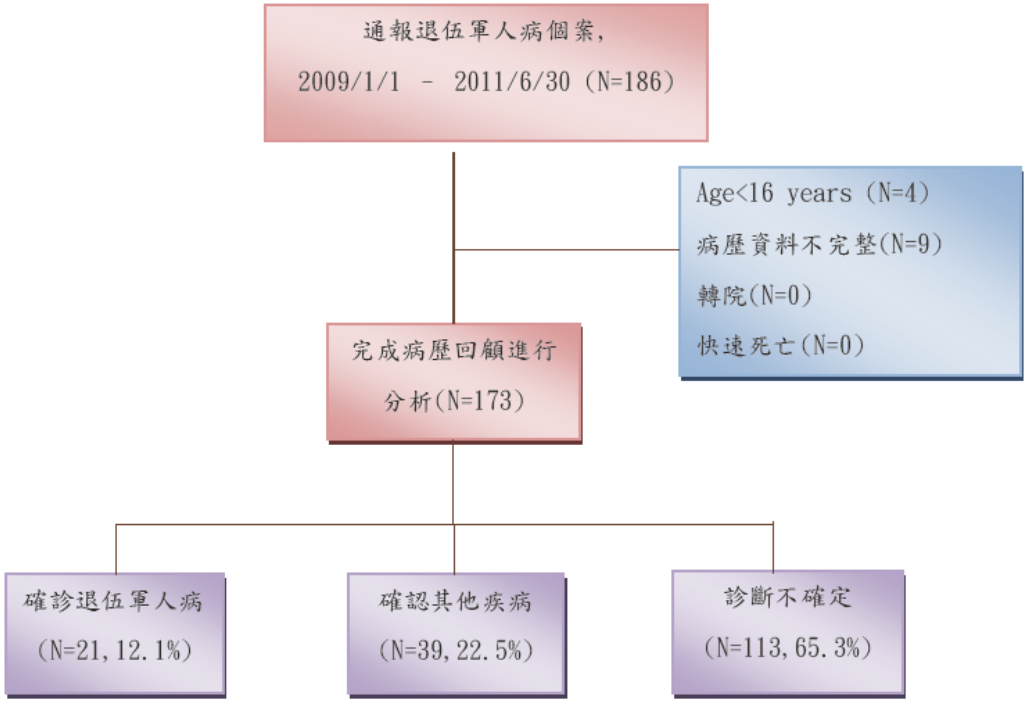
	NTUH	FEMH	KMUCMH	Total
Total No.	82	23	68	173
No. of patients confirmed with Legionellosis (%)	16 (19.5%)	3 (13.0%)	2 (2.9%)	21 (12.1%)
No. of patients without identified etiology (%)	47 (57.3%)	10 (43.5%)	56 (82.4%)	113 (65.3%)
No. of patients with possible etiology (%)	19 (23.2%)	10 (43.5%)	10 (14.7%)	39 (22.5%)
Mycobacterium tuberculosis (%)	3	0	1	4
Mycobacterium kansasii	1	0	0	1
Influenza A(sH1N1, H3N2, pH1N1) (%)	3	3	3	9
HSV1 (%)	1	0	0	1
CMV (%)	6	0	1	7
Adenovirus (%)	0	0	2	2
Human metapneumovirus (%)	0	0	1	1
Pnumocystic carnii	2	0	1	3
Endemic typhus (%)	1	0	0	1
Scrub typhus (%)	0	0	1	1
Pneumococcus (%)	2	0	0	2
CA-MRSA (%)	0	1	0	1
E. aerogenes (%)	0	1	0	1
H. parainfluenza (%)	0	1	0	1
P. aeruginosa (%)	0	1	0	1
S. marcescens (%)	1	0	0	1
E. coli (%)	1	0	0	1
Klebsiella pneumoniae (%)	0	1	0	1
Mycoplasma pneumoniae (%)	0	2	0	2
Chlamydia (%)	0	0	1	1

Figure 1. The retrospective study of 67 hospitalized patients with atypical pneumonia from 4 medical centers in Taiwan, 2009/1/1- 2009/12/31



- Confirmed cases with legionellosis
  - Compatible with case definition of legionellosis, Taiwan CDC
  - Confirmed, probable, or possible
- With any etiology other than Legionella identified
  - Confirmed with *Pneumocystis jirovecii,* Q fever, scrub typhus, endemic typhus, or leptospirosis by Taiwan,CDC
  - Positive results of sputum specimens by in house PCR or RT-PCR (13 virus panel), Taiwan CDC
  - Seroconversion for IgM, positive antigen detection, or PCR for Mycoplasma, Chlamydia, Pneumococcus, Aspergillus, Cryptococcus
  - a predominant organism isolated from multiple purulent sputums, and sputum quality was acceptable (WBC <10, neutrophil >25)
- No etiology identified
  - Negative findings from above mentioned tests

Figure 2. The retrospective study of 173 hospitalized patients with atypical pneumonia from 4 medical centers in Taiwan, 2009/1/1- 2011/06/30



附錄六

The Study of Emerging and Reemerging Infectious Diseases – *Pneumocystis jirovecii* pneumonia

Patients and Methods

Study site:

(1) National Taiwan University Hospital (NTUH)

(2) Far Eastern Memorial Hospital (FEMH)

(3) Kaohsiung Medical University Chung-Ho Memorial Hospital (KMUH)

Patients:

(1)≧16 years old;

(2) Pneumonia requiring hospitalization;

(3) Reported to Taiwan CDC for suspected *Pneumocystis jirovecii* pneumonia (PJP)

Data collection:

A standardized case report form including demographics: age, gender, underlying diseases, recent hospitalization (<90 days), residents of long-term care facility, date of admission, discharge, reported to CDC; diagnoses; complications: mechanical ventilation, ECMO support, renal replacment therapy; and outcome (in-hospital mortality).

Diagnosis of PJP

Confirmed cases was defined if *P. jirovecii* was identified by cytology of sputum or bronchoalveolar lavage, or histopathology of transbronchoscopic or surgical lung biopsy using traditional stains (Gomori methenamine silver, calcofluor white, etc.) or monoclonal antibodies. Probable cases was defined if the polymerase-chain reaction (PCR) assay for pneumocystis 16S rRNA of sputum or bronchoalveolar lavage was positive, plus a typical clinical history in high-risk patients, and either chest radiography that was consistent with interstitial pneumonitis (chest X-ray showing typical bilateral perihilar diffuse infiltrates, or ground-glass opacities by high-resolution computed tomography of the chest) or clinical response to anti- pneumocystis therapy. The definition was modified from Carmona EM &

Limper AH, Ther Adv Respir Dis 2011; 5(1): 41-59; Thomas CF, et al. N Engl J Med 2004;350:2487-98.

Results

A total of 196 episodes among 194 patients reported from July 2010 to June 2011 were analyzed which included NTUH 175 cases (among 173 patients), FEMH, 7 cases, and KMUH, 14 cases. Among them, 80 cases was PJP (confirmed cases: 2; probable cases: 78). Two patients had equivocal clinical manifestations, and improved clinically without anti-pneumocystosis therapy. Other etiologies, such as Mycoplasma, Cryptococcus and Aspergillus were identified.

Table 1 Clinical characteristics

	PJP (N=80)	Non-PJP (N=116)	*P* value
Age, year, mean ± SD	46 ± 14	56 ± 17	**<0.001**
16-44, year, N (%)	42 (52.5)	29 (25.0)	**<0.001**
45-64, year, N (%)	30 (37.5)	52 (44.8)	
≧65, year, N (%)	8 (10.0)	35 (30.2)	
Male sex, N (%)	60 (75.0)	64 (55.2)	**0.005**
Underlying diseases, N (%)			
HIV	35 (43.8)	10 (8.6)	**<0.001**
Hematological malignancy	22 (27.5)	39 (33.6)	0.363
Solid organ tumor	5 (5.3)	22 (19.0)	**0.011**
HSCT	6 (7.5)	10 (8.6)	0.778
Solid organ tranplantation	5 (6.3) Kidney: 3 Liver: 2	12 (10.3) Kidney: 10 Heart: 1 Lung: 1	0.317
Autoimmune disease	6 (7.5)	9 (7.8)	0.947
DM	8 (10.0)	20 (17.2)	0.154
HBV	18 (22.5)	11 (9.5)	**0.012**
HCV	3 (3.8)	1 (0.9)	0.160
Liver cirrhosis	1 (1.3)	2 (1.7)	0.790
CKD	5 (6.3)	8 (6.9)	0.858
ESRD	2 (2.5)	4 (3.4)	0.705
Cardiovascular diseases	3 (3.8)	19 (16.4)	**0.006**
Chronic lung diseases	2 (2.5)	8 (6.9)	0.169
Cerebral vascular diseases	1 (1.3)	3 (2.6)	0.516
Recent myelosuppresion, N (%)	10 (12.5)	21 (18.1)	0.291
Recent immunosuppression, N (%)	27 (33.8)	36 (31.0)	0.689
Neutropenia, N (%)	2 (2.5)	19 (16.4)	**0.002**
Hospital onset, N (%)	7 (8.8)	9 (7.8)	0.803
Community onset, with recent hospitalization (<90 days), N (%)	35 (35/73, 47.9)	52 (52/107, 48.6)	0.931
Mechanical ventilation, N (%)	31 (38.8)	67 (57.8)	**0.009**
ECMO, N (%)	1 (1.3)	6 (5.2)	0.146
AKI requiring RRT, N (%)	5 (6.3)	10 (8.6)	0.539
In-hospital mortality, N (%)	26 (32.5)	53 (46.6)	**0.049**
Morbidity, N (%)	5 (6.3)	10 (8.6)	0.539

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Table 2 Clinical characteristics between PJP cases with high or low titer of PCR

	High titer (++) (N=48)	Low titer (+) (N=29)	*P* value
Age, year, mean ± SD	44 ± 14	50 ± 14	**0.049**
16-44, year, N (%)	30 (62.5)	9 (31.0)	**0.023**
45-64, year, N (%)	15 (31.3)	15 (51.7)	
≧65, year, N (%)	3 (6.3)	5 (17.2)	
Male sex, N (%)	40 (83.3)	19 (65.5)	0.073
Underlying diseases, N (%)			
HIV	26 (54.2)	8 (27.6)	**0.023**
Hematological malignancy	10 (20.8)	12 (41.4)	0.053
Solid organ tumor	2 (4.2)	3 (10.3)	0.286
HSCT	1 (2.1)	5 (17.2)	**0.016**
Solid organ tranplantation	4 (8.3) Kidney: 2 Liver: 2	1 (3.4) Kidney: 1	0.399
Autoimmune disease	4 (8.3)	0	0.110
DM	4 (8.3)	4 (13.8)	0.447
HBV	13 (27.1)	5 (17.2)	0.323
HCV	0	3 (10.3)	**0.023**
Liver cirrhosis	0	1 (3.4)	0.195
CKD	1 (2.1)	3 (10.3)	0.113
ESRD	1 (2.1)	1 (3.4)	0.715
Cardiovascular diseases	2 (4.2)	1 (3.4)	0.875
Chronic lung diseases	1 (2.1)	1 (3.4)	0.715
Cerebral vascular diseases	1 (2.1)	0	0.434
Recent myelosuppresion, N (%)	6 (12.5)	4 (13.8)	0.870
Recent immunosuppression, N (%)	17 (35.4)	8 (27.6)	0.477
Neutropenia, N (%)	2 (4.2)	0	0.265
Hospital onset, N (%)	5 (10.4)	2 (6.9)	0.603
Community onset, with recent hospitalization (<90 days), N (%)	20 (20/43, 46.5)	13 (13/27, 48.1)	0.894
Mechanical ventilation, N (%)	17 (35.4)	14 (48.3)	0.265
ECMO, N (%)	1 (2.1)	0	0.434
AKI requiring RRT, N (%)	3 (6.3)	2 (6.9)	0.911
In-hospital mortality, N (%)	13 (23.1)	12 (41.4)	0.194
Morbidity, N (%)	2 (4.2)	3 (10.3)	0.286

There was no data available for 3 of 80 cases with confirmed PJP.

Table 3. Clinical outcomes in different populations

	Mechanical ventilation	ECMO	AKI requiring RRT	In-hospital mortality	Morbidity
Total (N=91)	46 (50.5)	2 (2.2)	7 (7.7)	34 (37.4)	6 (6.6)
HIV (N=23)	11 (47.8)	0	1 (4.3)	7 (30.3)	0
Hema.malignancy (N=29)	10 (34.5)	0	1 (3.4)	11 (37.9)	1 (3.4)
Solid organ tumor (N=11)	7 (63.6)	0	0	5 (45.5)	0
Solid organ Tx. (N=7)	3 (42.9)	0	1 (14.3)	1 (14.3)	1 (14.3)
Autoimmune dz. (N=9)	7 (77.8)	1 (11.1)	2 (22.2)	5 **(55.6)**	2 (22.2)
Others (N=17)	10 (58.8)	1 (5.9)	1 (5.9)	6 (35.3)	2 (11.8)

Table 4. The proportion of positive results determined by *RespiFinder®* among 91 patients with suspect PJP

	Positive results	Patients with possible etiologies	Patients with unknown etiology
Total (N=91)	36 (39.6)	19 (19/40, 47.5%)	17 (17/51, **33.3%**)
HIV (N=23)	9 (39.1)	9 (9/20, 45%)	0 (0/3, 0)
Hema. Malignancy (N=29)	10 (34.5)	3 (3/10, 30%)	7 (7/19, **36.8%**)
Solid organ tumor (N=11)	3 (27.3)	0 (0/0)	3 (3/11, **27.3%**)
Solid organ Tx. (N=7)	3 (42.9)	0 (0/0)	3 (3/7, **42.9%**)
Autoimmune dz. (N=9)	6 **(66.7)**	6 (6/8, 75%)	0 (0/1, 0)
Others (N=17)	8 (47.1)	1 (1/3, 33.3%)	6 (6/14, **42.9%**)

Of 40 patients with possible etiologies (determined by diagnostic tests based on routine practice or notifiable infectious diseases reporting system), 19 (47.5%) had one or more positive results based on RespiFinder as shown in the following: rhinovirus (8), Influenza A (3), parainfluenza 3 (3), Coronavirus 229E (1), RSV-A (1), RSV-B (1), human metapneumovirus (1), adenovirus + rhinovirus (1). That is, 5 of 40 cases (12.5%) had additional treatable etiologies identified (influenza, RSV). Of 51 patients with unknown etiology 17 (33.3%) had positive results: *Legionella pneumophila* (1), *Legionella pneumophila* + Rhinovirus (1), *Bordetella pertussis* + Coronavirus 229E (1), Human metapneumovirus (4), Rhinovirus (2), Coronavirus OC43 (2), Coronavirus 229E (1), Parainfluenza 4 (1), Influenza A (1), RSV-B (1), Coronavirus 229E + Human metapneumovirus (1), Coronavirus 229E + Parainfluenza 3(1). That is, 5 of 51 cases (10%) had treatable etiologies identified.

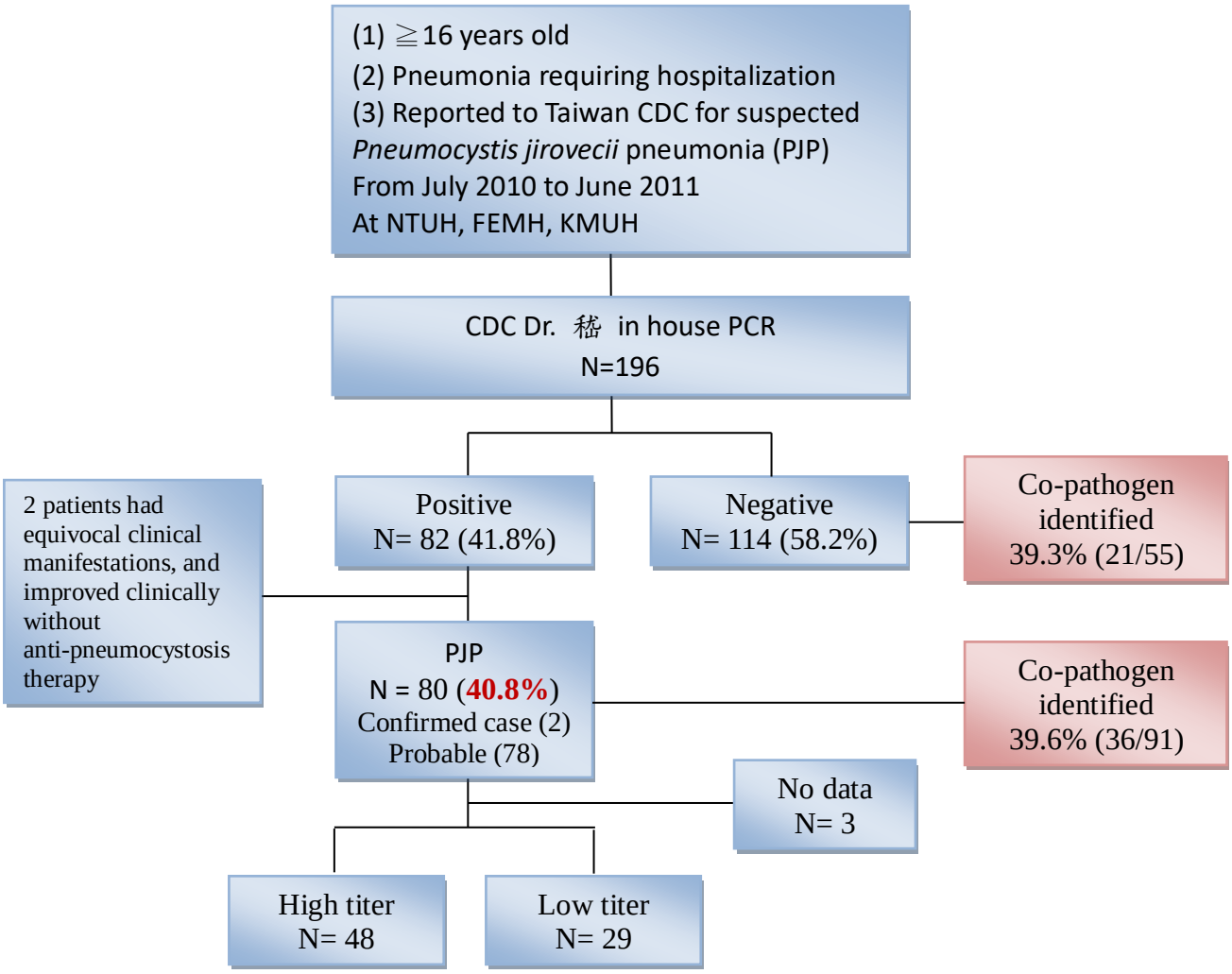

Figure 1. Patients with suspect *Pneumocystis jirovecii* pneumonia enrolled who received diagnostic tests based on routine practice, notifiable infectious diseases and *RespiFinder®* *19.* Two patients had equivocal clinical manifestations, and improved clinically without anti-pneumocystosis therapy.

附錄七

Detection of Acute Respiratory Viruses in Patients with Pneumonia with Unknown Etiology

Table 1. The proportion of positive results among 80 patients with pneumonia of unknown etiology enrolled from July 1, 2010 through Jun 30, 2011 determined by CDC Dr. Liu in house PCR and *RespiFinder® 19*

	NTUH	FEMH	KUMH	CSUH
All Subjects	14	55	8	3
Virus Detected by CDC in house PCR	2	25	2	1
RespiFinder	0	2	4	0
Total	2	27	4	1
Percentage of Positive Results	14.3%	49.1%	50%	33.3%

Note. Among 9 patients reported from NTUH 2010, 4 (46%) had one or more etiologies identied (參附錄四). However, data not completed yet.

Table 2. Distribution of viral etiologies among 80 patients with pneumonia of unknown etiology

	NTUH	FEMH	KUMH	CSUH	Total
Adenovirus		2	1*		3
CMV	1			1	2
HSV	1	1	1*		3
Influenza swH1N1		12 (2 mixed with B)	1		13 (2)
Influenza B		5 (2 mixed)			5 (2)
Influenza, other		1			1
parainfluenza		1			1
Coronavirus OC43		1	1		2
metapneumovirus		4	1		5
Rhinovirus		1			1
unknown		1			1
Overall	2	27	4	1	34

Table 3. Demographic, epidemiological, clnical characteristics and outcome of 80 patients evaluated

Parameter	All	Patients with Positive Viral Test
No. of pt	80	33[1] (34)
Gender (Male), %(no)	52.5% (42/80)	54.5% (18/33)
Mean Age	N/A	47.4
Admission (ER/OPD/Other)	57/6/9	27/3/3
Underlying diseases		
HIV infection	1.2% (1/80)	3.0% (1/33)
Hematologic Malignancy	1.2% (1/80)	3.0% (1/33)
Solid Organ Tumor	5.0% (4/80)	3.0% (1/33)
Hematopoietic stem cell transplantation	0	0
Solid organ transplantation	1.2% (1/80)	0
Recent myelosuppression therapy	0	0
Recent Immunosuppressant therapy	20.0% (16/80)	21.2% (7/33)
Neutropenia	1.2% (1/80)	0
Iron Overload	6.2% (5/80)	9.1% (3/33)
Antibiotic prophylaxix	65% (52/80)	78.8% (26/33)
Monoclonal antibody therapy	1.2% (1/80)	3.0% (1/33)
Autoimmune diseases	1.2% (1/80)	3.0% (1/33)
Diabetes mellitus	18.7 % (15/80)	27.3% (9/33)
HBV carrier	6.2% (5/80)	12.1% (4/33)
Chronic HCV infection	0	0

Liver Cirrhosis	1.2% (1/80)	0
Chronic kidney disease	8.7% (7/80)	9.1% (3/33)
ESRD	0	0
CVD	3(CHF), 34 (other)	4 (CHF), 12 (other)
Chronic Lung Disease	5 (COPD), 3 (asthma), 1 other	1 (COPD), 1(asthma)
CVA	8.7% (7/80)	15.1% (5/33)
Implant	1 (PPM), 1(valve), 1 (hip)	0
Epidemiological data		
Community	29	14
Nursing Home	2	1
RCW	1	0
HCAP	3	1
Travel History	4/80	1/33
Cluster	0	0
Animal Contact	2 (rodent), 2(dogs), 1 other	1 (dogs)
Family Hx	2 (TB), 6 (other)	1(TB), 4 (other)
Vaccination	1 (influenza)	1 (influenza)
Initial presentaion		
Fever	68.7% (55/80)	81.8% (27/33)
Altered consciousness	43.7% (35/80)	24.2% (8/33)
Constituinal symptoms	45.0% (36/80)	60.6% (20/33)
HEENT	7 (rhinorrhea), 12 (sore throat), 5 combined	3 (rhinorrhea), 7 (sore throat), 5 (combined)/33
Lymphadenopathy	0	0
Respiratory symptoms	37 (cough), 20 (other)	26 (cough), 3 (other)
CVS	7 (chest pain), 13 (dyspnea), 2 (chest pain + dyspnea), 1 other	4 (chest pain), 5 (dyspnea), 3 other
GI	31.2% (25/80)	69.6% (13/33)
GU	7.5 % (6/80)	3.0% (1/33)
Skin Lesions	2 (rash), 2(petechiae), 4 other	2 (petechiae), 2 other
Presence of co-pathogen		

Urinary Pneumococcal Ag	13.3% (2/15)	20% (1/5) * flu B
Chlamydia Ag	7.7% (1/13)	14.3% (1/7) * adenovirus
Mycoplasma 1st IgM	2.7% (1/39)	0
Mycoplasma 1st IgG	6/19	4/8 * HSV(1), flu (3)
Mycoplasma 2nd IgM	0	0
Mycoplasma 2nd IgG	0	0
Urine Legionella Ag	0	0
Cryptococcal Ag	0	0
Aspergillus Ag	0	0
Pathology	0	0
Outcome		
Respiratory failure requiring ventilator support	68.7% (55/80)	69.7% (23/33)
Respiratory failure requiring ECMO	12.5% (10/80)	9.1% (3/33)
Acute renal failure requiring hemodialysis	8.7% (7/80)	12.1% (4/33)
In Hospital Mortality	22.5% (18/80)	21.2% (7/33) 4 Flu, 2 HSV, 1 CMV

[1] One missing data

Main findings:

1. Influenza accounts half of the cases with one or more viral etiologies identified (17 cases), followed by metapneumovirus (5 cases), HSV (3 cases), and adenovirus (3). The clinical significance warranted further elucidated.
2. Of 7 patients died, 4 were influenza positive. This study also found that influenza vaccination rates were very low. Only one of 80 patients was vaccinated. Thus, diagnostic approach for viral etiologies might improve patient outcome.
3. All cases at FEMH were screened and reported by one of the authors according the diagnostic flowchart for viral pneumonia. The proportion of positive results was up to 50%. All of except one case with influenza were reported from this hospital. The data will be stratified into flu season or non-flu season. This finding demonated the difficulty in differential diagnosis of community-acquried pneumonia.

Discussion:

1. 單一病患有兩種以上病原體驗出時，何者為 true pathogen 的判定標準不易建立共識，應有其它定量分析/real-time PCR 或病理分析以協助判斷
2. 檢驗 viral load 的可能性？

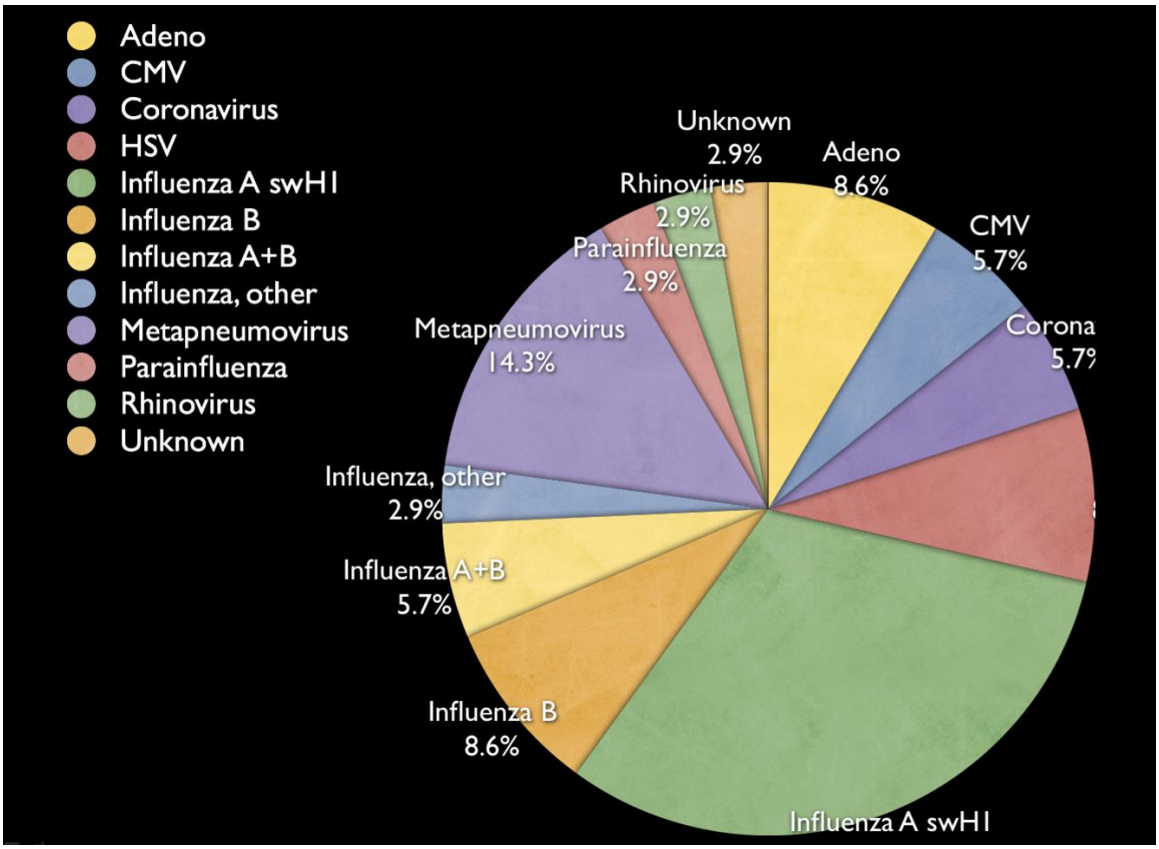

Figure 1. The distribution of viral etiologies among 80 patients determined by CDC Dr. Liu in house PCR and *RespiFinder® 19*

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細菌、黴菌及寄生蟲檢驗項目	台大	亞東	中山	高醫
Smear and stain				
Gram stain	+	+	+	+
Acid- fast bacilli (AFB) stain	+	+		+
Pneumocystis jiroveci stain (cytology)	+		+	+
India ink preparation	+			+
Parasite examination				
Malaria	+	+		+
Paragonimus ova	+			+
Amoebae	+	+		+
Giardia lamblia	+			+
Cryptosporidium & Isospora	+			+
Anal tape	+		+	
Parasite, others	+	+		+
Culture				
Salmonella/Shigella	+	+	+	+
Campylobacter	+	+	+	+
Other intestinal pathogen	+	+	+	+
Clostridium difficile	+	+	+	+
Aerobeic culture and identification	+	+	+	+
Anaerobic culture and identification	+	+		+
Fungus culture and identification	+	+		+
Histoplasma capsulatum[6]	+			+

Mycobacterium culture and identification	+	+		+
Culture for Acanthamoeba	+			
VRE survey[5]	+	+		
MRSA screening[5]	+	+		+(study)
Streptococcus pyogenes screening	+		+未分型	
H. pylori culture and identification	+	+		+
Gr B Strep.screening+drug susceptibility(自費)	+	+		+
N. gonorrhoeae identification	+	+		+
Serology				
S. pneumococcus antigen test	+	+	+	
S. pneumococcus antigen test (自費)[1]	+	+		
Legionella Ag	+	+		
Anti-Streptolysin O	+	+		+
group B streptococcal antigen test	+			+
Cryptococcal Ag	+	+	+	+
Aspergillus galactomanna Ag	+	外送	+	
Chlamydia Ag	+			+
Mycoplasma pneumoniae IgG	+	+	+	+
Mycoplasma pneumoniae IgM	+	+	+	+
S.T.S (TPPA)	+	+		+
S.T.S (VDRL/RPR)	+	+		+
Widal test & Weil-Felix test	+	+		+
***Candida* mannan Ag (BioRad)**[4]	+		+	

Candida anti-mannan Ab (BioRad)[4]	+		+	
Galatomannan Ag (BioRad) for *Aspergillus* and *Penicillium marneffei* [4]	+	外送	+	
Beta-D-glucan assay[4]	+		+	
Cryptococcal Ag[4]	+	+		+
***Histoplasma capsulatum* Ab[6]**	+		+	
***Coccidioides immitis*[6]**	+			
Rapid *H. pylori* antigen test	+		+	+
Amoeba, IHA	+	+		+
TB Quantiferon[3]	+			+
TB ELISPOT[2]	+		+	
Molecular diagnosis				
TB PCR(自費)	+	+	+	+
Western blot for HIV1, 2	+	+	+	+
BK virus	+		+	
CMV[7]	+	外送	+	+
***Mycoplasma* (自費)[1]**	+			
***C. trachomatis* Test (自費)**	+		+	
***N. gonorrhoeae* Test (自費)**	+			
Parvovirus[8]	+			+(自費)
***Pneumocystis jiroveci*[6]**	+			
Fungus[4,6]	+			
Others				
***C.difficile* toxin A+B test(自)**	+		+	+

說明 (自費、研究用、或防疫目的)

[1] 小兒部研究室 (分機 71728-71730)	[3] 王振源醫師研究室 (分機 62905)
PCR 送檢時間: 每週三, 週五中午以前	[4] 臨床感染症研究室 705 研究室(分機 65054)
PCR 查詢報告時間: 週二週五	[5] 感染管制研究室 730 研究室
PCR(定性): NTD 1500	[6] 疾病管制局合作實驗室
PCR(定量): NTD 2800	[7] 唐季祿醫師研究室
[2] 薛博仁醫師研究室 (手機 0966669577)	[8] 陳茂源醫師研究室