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وزارة التعليم العالي والبحث العلمي  
جامعة القادسية/كلية الطب  
فرع الاحياء المجهرية

تحديد الكائن الممرض ( *Pneumocystis jirovecii* )

في سائل غسل القصبات والحويصلات الهوائية والمسحات الانفيه في المرضى المثبتين مناعيا باستخدام  
المجهر والتقنيات الجزيئية

رساله مقدمه الى

مجلس كلية الطب في جامعة القادسيه

وهي جزء من متطلبات نيل درجة الماجستير في

الاحياء المجهرية الطبيه

تقدمت بها

مروة سلمان كاظم الجبوري

بكالوريوس طب وجراحه بيطريه (٢٠١٤)

بأشراف

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Republic of Iraq  
Ministry of Higher Education and Scientific Research  
University of Al-Qadisiyah / College of Medicine  
Department of Microbiology



# Identification of *Pneumocystis jirovecii* in Broncho Alveolar Lavage and Nasal Swabs of Immunocompromised Patients Using Microscope and Molecular Technique

A Thesis

Submitted to the Council of the College of Medicine / University of Al-Qadisiyah in partial Fulfillment of the Requirements for the master Degree of Science in Medical Microbiology

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

فَاعْلَمْ أَنَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ

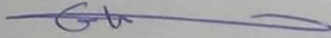
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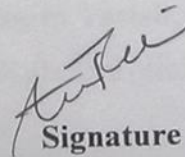
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## Supervisor's Certificate

We certify that this thesis entitled (**Identification of *Pneumocystis jirovecii* in broncho alveolar lavage and nasal swabs of immunocompromised patients using microscope and molecular technique**) was prepared under our supervision at the Department of Microbiology, College of Medicine , University of Al-Qadisiyah, as a partial fulfillment of the requirement for the degree of Master of Science in Medical Microbiology.



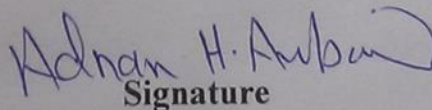
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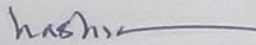
College of Medicine

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Date: 17/10 / 2016

## Certification of Examining Committee

We, the members of examining committee, certify after reading this thesis (Identification of *Pneumocystis jirovecii* in broncho alveolar lavage and nasal swabs of immunocompromised patients using microscope and molecular technique) and after examining the student **Marwah Salman Kadhim** in its contents, we found it is adequate for the degree of Master of science in Medical Microbiology.

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Chairman

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Professor

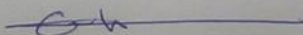
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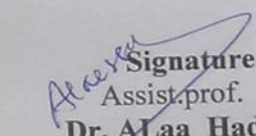
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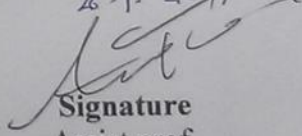
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Signature

Assist.prof.

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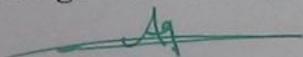
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Signature

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**Dr. Aqeel R. Hassan**

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30-1-2017

# **Dedication**

**To:**

**My Country Iraq ..... peace and safety**

**The spirit of my father**

**My dear mother**

**My brothers**

**Marwa Salman**



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**Marwa Salman**

## ***Summary***

---

The current study was designated by collecting of ninety-three samples, 53 samples were broncho alveolar lavage BAL, and 40 sample nasal swabs, BAL samples collected from Baghdad Medical City hospital and from Al-Diwaniyah teaching hospital while nasal swabs samples collected from Maternity and childhood teaching hospital in Al-Diwaniyah city at the period from the beginning of November 2015 to the end of May 2016. The present study included 93 patients, 40 (43%) infants and 53 (57%) adults. Mean age of infant patients was  $0.31 \pm 0.19$  months whereas the mean age of adult patients was  $44.25 \pm 16.16$  years and the current study included nasal swabs female 16 (40%) and male 24 (60%), BAL female 18 (34%) and the male 35 (66%)

*Pneumocystis jirovecii* were identified of microscopically in 38\53 from BAL samples only, the rate of infection with *pneumocystis jirovecii* according to microscopical diagnosis was 71.7%, the diagnosis of *pneumocystis jirovecii* were based on the morphology and diameter of cyst the mean diameter were  $0.61 \pm 0.07 \mu\text{m}$  with a range of 0.5- 0.77 $\mu\text{m}$

For the identification of *Pneumocystis jirovecii* in BAL and nasal swabs samples by using real-time PCR the result showed Positive samples were 72 and negative samples were 21, the rate of detection of *pneumocystis jirovecii* by qPCR method were 77.4%, the result showed that there was no significant difference in mean age of patients with positive qPCR than that with negative qPCR,  $24.02 \pm 25.14$  years versus  $29.88 \pm 24.65$  years ( $P=0.348$ ).

## ***Summary***

---

There were No significant association between gender of patients in nasal swabs and BAL with positive infection in *pneumocystis jirovecii* (P=0.681), (P=0.333), there was also no significant association between positive infection with *pneumocystis jirovecii* and residency of patients (P=0.515), the sensitivity, specificity, Positive predictive value and negative predictive value of microscopical examination were (87.2%, 71.4% ,89.5%, 66.7% ) respectively.

The following risk factors were identified: pneumonia in 56 (60.2%), COPD in single patient (1.1%), lung cancer in 7 (7.5%), TB in 9 (9.7 %), HIV in 1 (1.1%), renal transplantation in 5 (5.4%), asthma with steroid in 3 (3.2 %) patients, chronic liver disease in 5 (5.4%) and DM in 6 (6.5%).

Mean WBC count was not significantly different between positive and negative qPCR cases (P=0.599). Neutrophil, monocyte and lymphocyte percentages were significantly lower in patients with positive qPCR result (P $\leq$ 0.001), while Eosinophil percentage was significantly higher in positive cases (P <0.001).

DNA sequencing method was performed for Phylogenetic tree analysis study of local *Pneumocystis carinii* confirmative detection based mitochondrial large subunit ribosomal RNA gene, the local *Pneumocystis carinii* samples (S1,S2,S3,S4, and S5) were showed closed related to NCBI-Blast *Pneumocystis carinii* (KC494274.1) Whereas, the other NCBI-Blast *Pneumocystis* sp, were showed different and out of tree, The NCBI-Gene bank submission (S1:KX 901804,S2: KX 901805,S3: KX 901806,S4: KX 901807 and S5: KX 901808).

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## List of Abbreviations

Abbreviations	Meaning
AIDS	Acquired Immune Deficiency Syndrome
ABPA	Allergic bronchopulmonary aspergillosis
BAL	Broncho Alveolar Lavage
CDC	Centers for Disease Control
COPD	Chronic obstructive pulmonary disease
CF	Cystic fibrosis
DNA	Deoxyribonucleic acid
DHFR	Dihydrofolate reductase
GPA	Granulomatosis with polyangiitis
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
IL	Interleukins
ILDs	Interstitial lung diseases
LDH	Lactate dehydrogenase
MSG	Major surface glycoprotein
NCBI	National Center of Biotechnology Information
PJP	<i>Pneumocystis jirovecii</i> pneumonia
PCP	<i>Pneumocystis</i> pneumonia
PCR	polymerase chain reaction
RNA	Ribonucleic acid
RLL	Right lower lobe
SCID	Study combined immunodeficiency
EULAR	The European League Against Rheumatism
TS	Thymidylate synthase

Abbreviations	Meaning
WBC	White blood cell
USA	United States of America
UK	United Kingdom
TB	Tuberculosis
TBE	Tris/Borate/EDTA
TMP-SMX	Trimethoprim/sulfamethoxazole
SD	Standard deviation
RTX	Rituximab
RML	Right middle lobe
PPV	Positive predictive values
PJ	<i>Pneumocystis jirovecii</i>
NPV	Negative predictive values
MtolB	modified toluidine blue o stain
DLCO	Diffusing capacity of the lungs for carbon monoxide
DEPC	Diethylpyrocarbonate
DM	Diabetes mellitus
TMP-SMX	Trimethoprim-sulfamethoxazo
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
CT scan	Computed tomography
CXR	Chest x-ray
ANCA	Anti-neutrophil cytoplasmic antibodies
UPGMA	Unweighted Pair Group method with Arithmetic



## The Republic of Iraq Iraq ministry of health

Patient number:

Age:

Sex: male , or female

Geographical area : urban  Rural

### Case history:

1- Asthma Yes  or NO  2-Diabetes Yes , or No

3-Transplantation Yes , or No  4-pregnant Yes , or NO

5- Chemotherapy Yes , or NO  6- HIV Yes  or NO

7- Hepatitis YES  Or No

### blood tests:

WBCs count

### Symptoms:

---

1-

2-

3-

4-

5-

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in bronchoalveolar lavage  
fluid of immunocompromised patients  
using molecular technique  
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AUTHORS AL-Juborry,M.S., AL-Oumashi,G.B. and  
AL-Damarchi,A.T.  
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AUTHORS AL-Juborry,M.S., AL-Oumashi,G.B. and  
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College of Medicine,

University of Al-Qadissyia, Al-  
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Ascomycota; Taphrinomycotina;  
Pneumocystidomycetes;  
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JOURNAL Unpublished  
REFERENCE 2 (bases 1 to 256)  
AUTHORS AL-Juborry,M.S., AL-Oumashi,G.B. and  
AL-Damarchi,A.T.  
TITLE Direct Submission  
JOURNAL Submitted (25-SEP-2016) Microbiology,  
College of Medicine,  
University of Al-Qadissya, Al-  
Diwanyia street, Al-Diwanyia,  
Alqadissiya 00964, Iraq  
COMMENT GenBank staff is unable to verify  
sequence and/or annotation  
provided by the submitter.



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EMAIL:hassan_iq84@yahoo.com.
Bankit Comment: TOTAL # OF SEQS:5.
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 Ascomycota; Taphrinomycotina;  
 Pneumocystidomycetes;  
 Pneumocystidaceae; *Pneumocystis*.  
 REFERENCE 1 (bases 1 to 253)  
 AUTHORS AL-Juborry,M.S., AL-Oumashi,G.B. and  
 AL-Damarchi,A.T.  
 TITLE Identification *Pneumocystis jirovecii*  
 in bronchoalveolar lavage  
 fluid of immunocompromised patients  
 using molecular technique  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 253)  
 AUTHORS AL-Juborry,M.S., AL-Oumashi,G.B. and  
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VERSION
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Ascomycota; Taphrinomycotina;
            Pneumocystidomycetes;
Pneumocystidaceae; Pneumocystis.
REFERENCE  1  (bases 1 to 255)
    AUTHORS  AL-Juborry,M.S., AL-Oumashi,G.B. and
AL-Damarchi,A.T.
    TITLE    Identification Pneumocystis jirovecii
in bronchoalveolar ravage
            fluid of immunocompromised patients
using molecular technique
    JOURNAL  Unpublished
REFERENCE  2  (bases 1 to 255)
    AUTHORS  AL-Juborry,M.S., AL-Oumashi,G.B. and
AL-Damarchi,A.T.
    TITLE    Direct Submission
    JOURNAL  Submitted (25-SEP-2016) Microbiology,
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University of Al-Qadissyia, Al-Diwanyia  
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EMAIL:hassan\_iq84@yahoo.com.

Bankit Comment: TOTAL # OF SEQS:5.

Bankit Comment: TOTAL # OF SETS:5.

##Assembly-Data-START##

Sequencing Technology :: Sanger

dideoxy sequencing

##Assembly-Data-END##

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صممت الدراسة الحاليه بواسطه جمع 93 عينه 40 عينه كانت مسحه انفيه و53 عينه كانت سائل غسل القصبات والحويصلات الهوائيه , عينات سائل غسل القصبات والحويصلات الهوائيه جمعت من مستشفى مدينة الطب بغداد ومن مستشفى الديوانيه التعليمي بينما عينات المسحه الانفيه جمعت من مستشفى الاطفال في مدينة الديوانيه والعينات جمعت عند الفتره من بداية شهر نوفمبر 2015 الى نهاية شهر مايو 2016 عينات الدراسه تضم نسبة (43%) 40 اطفال ونسبة (57%) 53 بالغين, معدل اعمار المرضى الاطفال كان  $0.31 \pm 0.19$  شهر بينما معدل اعمار المرضى البالغين كان  $44.25 \pm 16.16$  سنه.

تحديد الكائن الممرض *Pneumocystis jirovecii* بواسطه المجهر الضوئي في 38\53 كان في عينات سائل غسل القصبات والحويصلات الهوائيه فقط معدل الاصابه بالكائن الممرض طبقا الى التشخيص المجهرى كانت 71.7% ومعدل حجم الكائن الممرض كان  $0.07 \pm 0.61$  مايكرومتر مع معدل 0.5-0.77 مايكرومتر.

لغرض تحديد *Pneumocystis jirovecii* في عينات سائل غسل القصبات والحويصلات الهوائيه والمسحه الانفيه بواسطه استخدام real-time PCR النتائج اظهرت العينات الموجبه 72 والعينات السالبه 21 معدل تحديد *pneumocystis jirovecii* كان محسوب بمعدل 77.4%.

كذلك لاتوجد ارتباطات مميزه بين الاصابه الموجبه مع *pneumocystis jirovecii* واماكن سكن المرضى ( $P=0.515$ ) , حساسيه التشخيص المجهرى , الخصوصيه, قيم التنبؤ الموجبه وقيم التنبؤ السالبه) (87.2%, 71.4%, 89.5%, 66.7%).

## الخلاصة

عوامل الخطوره التاليه التي حددت:مرض ذات الرئه في 56 مريض (60.2%) , امراض الرئه الانسداده المزمنه في مريض واحد (1.1%), سرطان الرئه في 7 مرضى (7.5%) , السل الرئوي في 9 مرضى (9.7%), مرض الايدز في مريض واحد (1.1%), نقل الكلى في 5 مرضى (5.3%), مرض الربو في 3 مرضى (3.2%), مرض التهاب الكبد الفيروسي في 5 مرضى (5.4%), ومرض السكري في 6 مرضى (6.5%).

معدل خلايا الدم البيضاء كانت لاتحمل فروقات مميزه بين النتيجة الموجبه والسالبه للفحص بواسطه qPCR (P=0.599) نسب خلايا الدم وهي الخليه المعتدله والخليه الاحاديه والخليه البلعيه (Neutrophil, monocyte and lymphocyte) كانت منخفضه انخفاض مميز مع النتيجة الموجبه للفحص بواسطه qPCR result (P<0.001) بينما نسبة الخلايا الخليه الحمضه Eosinophil كانت ارتفاعها مميز في الحالات الموجبه. (P<0.001)

طريقة تتابع الحمض النووي DNA كانت منجزه لغرض تحليل دراسة الشجره الجينيه للكائن الممرض *Pneumocystis carinii* والتحديد التثبتي كان يعتمد على وحدات الريبوسومات المايتركرييه الكبيره لجينات RNA الكائن الممرض المحلي *Pneumocystis carinii* عينات او عزلات الخمسه (S1,S2,S3,S4, and S5) اظهرت علاقه مغلقه الى العزله العالميه NCBI-Blast *Pneumocystis carinii* (KC494274.1) في حين *Pneumocystis sp.* NCBI-Blast الاخرى كانت اظهرت اختلاف خارج الشجره وشفرات العينات المسجله في بنك الجينات العالمي هي:

The NCBI-Gene bank submission (S1:KX 901804,S2: KX 901805,S3: KX 901806,S4: KX 901807 and S5: KX 901808).