MEMORANDUM FOR SGVU
ATTN: THOMAS GIBBONS

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled *The Influenza Virus and the 2009 H1N1 Outbreak* presented at *2016 Texas Association for Clinical Laboratory Science Conference, San Antonio, TX 6-8 April 2016* with MDWI 41-108, and has been assigned local file #16146.

2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.

3. Please know that if you are a Graduate Health Sciences Education student and your department has told you they cannot fund your publication, the 59th Clinical Research Division may pay for your basic journal publishing charges (to include costs for tables and black and white photos). We cannot pay for reprints. If you are 59 MDW staff member, we can forward your request for funds to the designated wing POC.

4. Congratulations, and thank you for your efforts and time. Your contributions are vital to the medical mission. We look forward to assisting you in your future publication/presentation efforts.

LINDA STEEL-GOODWIN, Col, USAF, BSC
Director, Clinical Investigations & Research Support

Warrior Medics – Mission Ready – Patient Focused
TO: Clinical Research Division/SGVU (59 MDW/SGVU)
FROM: Author's Name, Rank, Grade, Office Symbol
Thomas Gibbons, GS-14, 59 MDW/SGVUL

PROTOCOL NUMBER:
N/A

PROTOCOL TITLE: [NOTE: For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.]

Educational Presentation

1. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED
The Influenza Virus and the 2009 H1N1 Outbreak

2. FUNDING RECEIVED FOR THIS STUDY? ☐ YES ☒ NO 
FUNDING SOURCE: [ ]

3. IS THIS MATERIAL CLASSIFIED? ☐ YES ☒ NO

4. IS THIS MATERIAL SUBJECT TO ANY LEGAL RESTRICTIONS FOR PUBLICATION OR PRESENTATION THROUGH A COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA), MATERIAL TRANSFER AGREEMENT (MTA), INTELLECTUAL PROPERTY RIGHTS AGREEMENT ETC.? ☐ YES ☒ NO

NOTE: If the answer is "YES" then attach a copy to the Agreement to the Publications/Presentations Request Form.

5. MATERIAL IS FOR (Check appropriate box or boxes for approval with this request.)
[ ] DOMESTIC RELEASE [ ] FOREIGN RELEASE

- PUBLICATION/JOURNAL (List intended publication/journal)
- PUBLICATION ABSTRACT (List intended journal.)
- POSTER (To be demonstrated at meeting/Name of Meeting, City, State, and Date of Meeting)
- PLATFORM PRESENTATION (At civilian institutions/Name of Meeting, State, Date of Meeting)
- 2016 Texas Association For Clinical Laboratory Science Conference, San Antonio Texas, April 6-8 2016.
- OTHER (Describe: Name of Meeting, City, State, and Date of Meeting)

6. WHAT IS THE EXPECTED DATE YOUR PRESENTATION/PUBLICATION WILL BE SUBMITTED TO THE DEFENSE TECHNICAL INFORMATION CENTER (DTIC)?

7. WHO IS THE PRIMARY 59 MDW POINT OF CONTACT? (Last, First, Ml.) (Include email)
Gibbons, Thomas, F. thomas.gibbons.4@us.af.mil

DUTY PHONE/PAGER No.
210-292-7363

AUTHORSHIP AND CO-AUTHOR(S) (List in the order they will appear in the manuscript)

<table>
<thead>
<tr>
<th>LAST NAME</th>
<th>FIRST NAME AND Ml.</th>
<th>GRADE/RANK</th>
<th>SQUADRON/GROUP/OFFICE SYMBOL</th>
<th>INSTITUTION (If not 59 MDW)</th>
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<tbody>
<tr>
<td>a.</td>
<td>Primary/corresponding author</td>
<td>GS-14</td>
<td>59 MDW/SGVUL</td>
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<tr>
<td>b.</td>
<td>Gibbons, Thomas F.</td>
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I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401.1 AND 59 MDW 41-108. I HAVE READ THE FINAL VERSION OF THE ATTACHED MATERIAL AND CERTIFY THAT IT IS AN ACCURATE MANUSCRIPT FOR PUBLICATION AND/OR PRESENTATION.

AUTHOR'S PRINTED NAME/RANK/GRADE
Thomas F. Gibbons GS-14

AUTHOR'S SIGNATURE
GIBBONS,THOMAS.F.1128005281
Date: Mar 28, 2016

APPROVING AUTHORITY'S PRINTED NAME, RANK, TITLE
Paul T. Barnicott GS-15

APPROVING AUTHORITY'S SIGNATURE
BARNICOTT,PAUL.T.1102319273
Date: Mar 29, 2016
# PROCESSING OF PROFESSIONAL MEDICAL RESEARCH PUBLICATIONS/PRESENTATIONS

## 1st INidoresMENT (SGVU Use Only)

<table>
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<tr>
<th>TO:</th>
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<th>30 Mar 2016</th>
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| 4. DATE FORWARDED TO PA | |
|-------------------------| |

| 5. AUTHOR CONTACTED FOR RECOMMENDED OR NECESSARY CHANGES | 
|---------------|--------------|

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<th>NO</th>
<th>YES (if yes give date)</th>
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<th>6. COMMENTS</th>
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- APPROVED
- DISAPPROVED

The presentation is approved. This will require a JA Ethics review.

## 2nd INidoresMENT (PA Use Only)

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- APPROVED (In compliance with security and policy review directives.)
- DISAPPROVED

## 3rd INidoresMENT (SGVU Use Only)

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<th>59 MDW/SGVU</th>
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| 1. DATE RECEIVED | |
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<th>2. SENIOR AUTHOR NOTIFIED BY PHONE OF APPROVAL OR DISAPPROVAL:</th>
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- YES
- NO
- Could not be reached
- Left message

| 3. DATE WRITTEN NOTICE OF APPROVAL AND CLEARANCE MAILED TO AUTHOR: | |
|-----------------------------------------------------------------| |

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<th>4. COMMENTS</th>
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- APPROVED
- DISAPPROVED

## Printed Name, Rank/grade, Title of Reviewer

<table>
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<th>Rocky Calcote, PhD, Clinical Research Administrator</th>
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|CALCOTE.ROCKY.D.1178245844|

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Supersedes 59 MDW Form 3039, 20131018
MEMORANDUM FOR 59 MDW/SGUV

FROM: 502 ISG/JAC

SUBJECT: Ethics Review for Presentation Approval Request

1. The poster presentation titled “The Influenza Virus and the 2009 H1N1 Outbreak” has been submitted for legal review. There are no apparent conflicts of interests and the poster presentation includes the required disclaimer. This poster presentation may be submitted for exhibition at conference.

2. FACTS: The poster presentation titled “The Influenza Virus and the 2009 H1N1 Outbreak” was submitted by Dr. Thomas Gibbons of 59 MDW/SGUV. Dr. Gibbons plans to present this poster presentation at the 2016 Texas Association for Clinic Laboratory Science Conference in San Antonio, Texas from 6 – 8 April 2016.

3. LAWS AND REGULATIONS: DoD 5500.07-R, Joint Ethics Regulation (JER), section 3-305 lays out rules governing “Teaching, Speaking and Writing.” If the abstract will include the presenter’s rank or title and “deal in significant part with any ongoing or announced policy, program or operation” of the Air Force, the presenter is required to include a disclaimer that states the “views presented are those of the speaker or author and do not necessarily represent the views of DoD or its Components.”

4. ANALYSIS: While this poster presentation does not deal “in significant part with any ongoing or announced policy, program or operation” of the Air Force, the presenter’s affiliation and/or rank will nevertheless be included in the poster presentation. Accordingly, the disclaimer is required here and Dr. Gibbons has properly included it. A Public Affairs review will be needed if it has not already been obtained. There are no apparent conflicts of interest that would prohibit presentation of this information at this professional meeting.

5. CONCLUSION: The poster presentation presented for review included the disclaimer required by the JER. There do not appear to be any conflicts of interest. If you have any questions, please call the undersigned at 671-3362.

MARK E. COON, Major, USAF
Acting Chief, Civil Law
The Influenza Virus and the 2009 H1N1 Outbreak

Thomas. F. Gibbons, Ph.D.
Chief, Laboratory Services Branch
Clinical Research Division
Wilford Hall Ambulatory Surgical Center

The views expressed are those of the presenter and do not reflect the official policy of the Department of Defense or its Components.
Overview

• “The Story”
• Key aspects of the influenza virus
• Laboratory techniques utilized to discover the 2009 H1N1 influenza virus
• Laboratory techniques instrumental to influenza surveillance/vaccine contents
Video depicting USAF “Epi lab’s” discovery of H1N1

April 1 2009  Video team comes to our lab to film a documentary on our role with respect to influenza surveillance

http://www.ndep.us/Tracking-the-Flu

April 16 2009 “Found a weird flu virus today”
USAFSAM Influenza Surveillance during the off season: April 2009 – October 2009
By: Thomas F. Gibbons, Maj, USAF, BSC, Ph.D, MT (ASCP)
Chief, Molecular Diagnostics, USAFSAM

Influenza virus is a segmented negative stranded RNA virus composed of 8 gene segments belonging to the Orthomyxoviridae family. Three types of influenza have been detected, A, B and C, two of which (A and B) are a

http://www.safmls.org/society_scope.html
Key aspects of the influenza virus

Types of influenza

A, B, and C (Epidemics = A & B)
Pandemics = A only

1918 = H1N1
50,000,000 deaths

1957 = H2N2
1,000,000 deaths

1968 = H3N2
500,000 deaths

Shift and Drift

Annual flu
250,000 deaths

2009 H1N1

H5N1 annual
25 - 75
Key aspects of the influenza virus

**Influenza Genome**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Gene</th>
<th>Length (bp)</th>
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<tr>
<td>1</td>
<td>PB2</td>
<td>2341</td>
</tr>
<tr>
<td>2</td>
<td>PB1</td>
<td>2341</td>
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<tr>
<td>3</td>
<td>PA</td>
<td>2233</td>
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<td>4</td>
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<td>1778</td>
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<tr>
<td>5</td>
<td>NP</td>
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<td>6</td>
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</tr>
<tr>
<td>8</td>
<td>NS</td>
<td>890</td>
</tr>
</tbody>
</table>
Key aspects of the influenza virus

**Influenza**

- Nucleoprotein (RNA)
- Lipid Envelope
- Capsid
- Hemagglutinin
- Neuraminidase (Sialidase)

Figure 1


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<thead>
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<th>Segment</th>
<th>Size (bp)</th>
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<tr>
<td>1</td>
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Key aspects of the influenza virus

Influenza Life Cycle

1: Binding and entry
2: Release of vRNPs
3: RNA replication
4: RNA translation
5: Protein transport
6: Viral transport
7: Viral budding and release

Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

- Lateral Flow assay
- Culture
- PCR
- Sequencing
Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus
Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

Matrix Gene

Hemagglutinin Gene
Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

A/California/04/2009 (H1N1)

PB2: Swine 97.6%, Duck 97.2%, Human 96.8%
PB1: Swine 97.0%, Turkey 96.6%
PA: Swine 96.0%, Human 95.8%
HA: Swine 96.4%, Turkey 96.2%
NP: Swine 97.8%
NA: Swine 93.6%
MA: Swine 98.2%
NS: Swine 95.6%

What is the swine flu like?

Blast analysis results for a recent “Swine Flu”

Blast results say “Swine”

but it is infecting humans

Blast comparison of first 500 nucleotides for each segment
Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

A/Washington/AF06/2007 (H1N1)

PB2: Human 99.8%
PB1: Human 100%
PA: Human 99.6%
HA: Human 99.6%
NP: Human 99.6%
NA: Human 99.8%
MA: Human 100%
NS: Human 100%

What is the human H1N1 flu like?

blast analysis results for one of the recent "Human Flu"

Blast comparison of first 500 nucleotides for each segment
Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

Gene by gene comparison of "Swine" H1N1 with "Human" H1N1

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<td>82.7%</td>
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</tr>
<tr>
<td>NS</td>
<td>81.7%</td>
<td>NS</td>
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Key laboratory techniques utilized to discover the 2009 H1N1 influenza virus

**Phylogenetic Tree**

Out of the over 400 human H1’s USAFSAM sequenced this season no specimen has had less than a 98.2% homology with respect to the HA1 region of HA.

Swine H1N1 has about 73% homology with respect to the current vaccine for H1N1 (anything below 97% is a preliminary predictive factor for a strain that the vaccine will not protect against.)

73% homology to A/Brisbane/59/2007
Key laboratory techniques instrumental to influenza surveillance/vaccine contents

• Contents of the annual Flu Vaccine
  - It is recommended that trivalent vaccines for use in the 2016-2017 northern hemisphere influenza season contain:
    - A/California/7/2009 (H1N1)pdm09-like virus;
    - A/Hong Kong/4801/2014 (H3N2)-like virus;
    - B/Brisbane/60/2008-like virus.
    - It is recommended that quadrivalent vaccines containing two influenza B viruses contain the above three viruses and a B/Phuket/3073/2013-like virus
Key laboratory techniques instrumental to influenza surveillance/vaccine contents

<table>
<thead>
<tr>
<th>Host of origin</th>
<th>Geographic location of first isolation/strain</th>
<th>Number/year of isolation</th>
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</thead>
<tbody>
<tr>
<td>A/New York</td>
<td>11/2003</td>
<td>(H3N2)</td>
</tr>
<tr>
<td>A/chicken/Indonesia</td>
<td>R132-134/03</td>
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</tr>
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</table>

(By convention, the host of origin of human strains is not included)
Key laboratory techniques instrumental to influenza surveillance/vaccine contents

- Shot Versus Flu Mist
- How does Tamiflu work
- Sequencing HA
  - Culture, HAI, PCR, Serology
Surveillance for a new Virus

A/North Carolina/5475/2010: 98.2% overall HA1 amino acid homology

- V47E (valine to glutamic acid)
- P83S (proline to serine)
- D97N (aspartic acid to asparagine)
- S203T (serine to threonine)
- M257V (methionine to valine)
- I321V (isoleucine to valine)

Built using first approach mode in Swiss Model. Utilized amino acids from vaccine strain and viewed in Swiss-PdbViewer with amino acids differing from vaccine strain color coded red.
2015-2016 Report 4 Influenza A(H1N1)pdm09
HA Phylogenetic Analysis

Figure 1

2015-2016 A(H1N1)pdm09 Vaccine strain: A/California/07/2009
Reference Strain
October 2015
December 2015
January 2016
February 2016

ADD GLY Create Glycosylation Motif
F CDC Reference Antigen
e Egg Isolate
ORES Resistance to Oseltamivir
2015-2016 Report 4 Influenza A(H3N2) 
HA Phylogenetic Analysis

Figure 2

2015-2016 A(H3N2) Vaccine strain: A/Switzerland/9715293/2013
Reference Strain
November 2015
December 2015
January 2016
February 2016
ADD GLY Create Glycosylation Motif
LOSS GLY Loss of Glycosylation Motif
F CDC Reference Antigen
e Egg Isolate
LR Low Reactor to: A/Texas/50/2012 (28 fold)

2015-2016 A(H3N2) Phylogenetic Tree

- A/California/1570/2016
  - A/California/NHRC27575/2015
  - A/California/NHRC27568/2015
  - A/California/NHRC27574/2015
  - A/California/NHRC27577/2015
  - A/California/NHRC27582/2016

- A/California/NHRCBRD41174N/2016
  - A/California/NHRC528AR20825N/2015
  - A/Maryland/28/2014 LR F
    - A/Canberra/82/2014
      - A/Louisiana/20/2014 LR F
  - A/South Korea/x9/2015

- A/New York/39/2012 e F
  - A/Texas/1732/2016
    - A/California/02/2014 LR F
      - A/Switzerland/9715293/2013 e LR F

- A/Texas/50/2012 e F
  - A/Victoria/361/2011 e F
Questions?
References not previously listed:

- Slide 23 & 24
- Slide 25 & 26
  Influenza Research Database (www.fludb.org), a federally funded project supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, Contract No. NIH N01AI 400041