Philippine Society of Pathologists, Inc.

Vol. 2 No. 2 November 2017
ISSN 2507-8364 (Online)

PHILIPPINE JOURNAL OF PATHOLOGY

The Official Journal of the Philippine Society of Pathologists, Inc.

IN THIS ISSUE:

Philippine Journal of Pathology
Vol. 2 No. 2 November 2017 | ISSN 2507-8364 (Online)
http://philippinejournalofpathology.org

PHILIPPINE JOURNAL OF PATHOLOGY

The Philippine Journal of Pathology (PJP) is an open-access, peer-reviewed, English language, medical science journal published by the Philippine Society of Pathologists, Inc. Committee on Publications. It shall serve as the official platform for publication of high quality original articles, case reports or series, feature articles, and editorials covering topics on clinical and anatomic pathology, laboratory medicine and medical technology, diagnostics, laboratory biosafety and biosecurity, as well as laboratory quality assurance. The journal's primary target audience are laboratorians, diagnosticians, laboratory managers, pathologists, medical technologists, and all other medical and scientific disciplines interfacing with the laboratory. The PJP follows the **ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, EQUATOR Network Guidelines,** and **COPE Guidelines.** The PJP does not charge any article processing or submission fees from authors. It does not charge any subscription fees or download fees to access content.

To all our PJP readers,

It gives me immense pride and enthusiasm that I invite you to read our third issue and the succeeding issues of the Philippine Journal of Pathology.

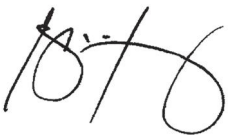
After quite a while, It was only three years ago that we have resumed the talk on publishing our own society's journal and once it was approved even if we knew it will be difficult, we proceeded with the publication of our journal. Enormous brain storming and effort was done to come up with this journal and I believe you now see that effort reflected in each edition and in the impact it will have on the field of Pathology.

Our journey to the birth of this journal is not smooth considering the cost of publication plus the difficulty of acquiring articles to be published. The journal intends to publish case reports, review articles and most espiecially original research articles. Our objective is to reach all the pathologists and clinical practitioners, who are interested in pathology's interesting cases, research activities and probably new techniques which helps us in updating our knowledge.

The PSP together with the PJP's editorial staff welcome you to post comments related to the journal by sparing your valuable time and request you to send articles.

Finally I would like to thank the PJP editorial team headed by Dr. Mads Tandoc, his technical team, the authors and the well wishers, who are promoting this journal. We maintain that the standard policies will be followed and we remain open to comments and suggestions but most of all, we are most open to your continued support.

Thank you!



Bernadette R. Espiritu, M.D. FPSP. MMHoA. MIAC
President, Philippine Society of Pathologists, Inc.

Horror Vacui

Each completed issue of the PJP is no small feat. The editorial process is not as simple as receiving articles, laying them out and sending them to press. Additional processes, based on international standards, are now in place in between point of submission and final publication, to assure quality: a checklist of requirements and forms need to be submitted at the outset; a review of statistical methods, if applicable, need to be hurdled; a blind peer review system must be completed to help the editor arrive at a decision to either accept or reject a submitted manuscript; and then, there is the back and forth communication between author and editor, to ensure that suggested changes are discussed and considered. It does not end there. Between author resubmission to final publication lie copyediting and layout, incorporation of digital object identifiers, and PDF and HTML conversion.

We aim to assure our readers that every article you have read in the past issues since our journal's revival, and those you shall read in this issue and future issues, is quality controlled.

There exists a large expanse of time and space between journal issues released only during conventions. We try to address this vacuum by aiming for a second issue within the year. While article submissions may be lean for now, the fact that we have reached a second issue for 2017 alone is, in itself, already a milestone. It is something that we should be proud of and hope we could sustain in 2018 and beyond.

Content-wise, policy-guiding research from two National Reference Laboratories are included in this issue. Data generated from the national external quality assessment of blood service facilities for 2016 reinforce the need for participants to proactively review their EQAS reports, discuss and implement corrective actions and opportunities for improvement with their staff and management. Seven-year data from 2009 to 2015 of the external quality assurance scheme for bacteriology show that, although there is, in general, improvement of the performance of tertiary clinical laboratories, there are still poorly performing facilities lacking trained personnel, resources, and implementation of quality assurance, that need attention. We hope that the other National Reference Laboratories shall be encouraged to follow suit in the upcoming issues, as NRL outputs are critical to fill in the gaps in national laboratory policies.

This second issue now also features representative articles for the "Review" and "Autopsy Vault" sections. The article by Bajpai and Pardhe proposes a working classification for oral neoplasms with basaloid morphology. The submission by Lo and Lique discusses a clinical enigma whose rare cause was solved postmortem. Both provide important learning points for pathologists and diagnosticians.

Moreover, we are happy to introduce "Diagnostic Perspectives," a new type of article for the PJP with characteristics in between a feature article, a case report, and images in pathology. Through this, we aim to feature new technologies and innovations that improve diagnostics and ultimately, clinical management. Preliminary findings, proofs-of-concept, and scientific anecdotes, will find good company in this new section. In this issue, we feature two such articles, one on a novel functional imaging modality for breast cancer correlated with histopathologic findings, and another, on the use of patented magnetic nanoparticles for improving sensitivity of pathogen detection.

Tilbe *et al.*'s work on cases of inflammatory bowel disease seen in a local tertiary hospital, and Villanueva *et al.*'s report of a rare variant of a commonly seen pathologic entity, underscore the enduring value of the case report, as a learning resource. Rounding up this issue, is the experimental work by Dematera *et al.* to provide additional objective data on a classic test which, although it may already have been superseded by technology, is still being used in our setting.

"*Horror vacui,*" ("Nature abhors vacuum,") so said Aristotle, in Book IV of his treatise, *Physics*. In the modern context, meaning, if there are gaps, something shall fill them. It is our hope that the Philippine Society of Pathologists and its members will use the Philippine Journal of Pathology as an instrument in solidifying the evidence basis for national laboratory policies and, in the process, fill the gaps in local data.

Amado O. Tandoc III, MD, FPSP
Editor-in-Chief

https://doi.org/10.21141/PJP.2017.010

Invitations from the

BioRisk Association of the Philippines, Inc.

For inquiries, pls call +63.9292.660

** This advertisement is a complimentary service of the PJP for member societies/organizations.*

OPEN ACCESS – ORIGINAL ARTICLE

The Effect of Number of Tests, Hemoglobin Level and Working Temperature on the Specific Gravity of 100 ml Copper Sulphate Solution in Hemoglobin Screening

Francis Dematera and Linda Tamesis

Institute of Medicine, Far Eastern University-Nicanor Reyes Medical Foundation

ABSTRACT

Background. The copper sulphate method, being economical, was used for hemoglobin screening in blood donation. Various references cite different number of tests that can be performed in a 100 mL copper sulphate solution.

Objective. To determine the effect of the number of tests, hemoglobin level and working temperature on the specific gravity of 100ml copper sulfate solution.

Methodology. Three groups of samples of known hemoglobin levels (<12 g/dl, 12-14 g/dl, and >14 g/dl) were tested using a 100 ml copper sulphate solution with specific gravity 1.053 at room temperature and at temperature of 29-30°C. Specific gravity of the solution was measured after every 5 tests for a total of 50 tests per experiment.

Result. There was no change in the specific gravity of copper sulphate solution used in 50 tests. There was no difference in the measured specific gravity across all experiments.

Conclusion. A 100 mL copper sulphate solution can be used for 50 tests using samples of various hemoglobin levels, at room temperature and at a higher temperature.

Key words: blood, hemoglobin, copper sulphate

ISSN 2507-8364 (Online)
Printed in the Philippines

Received: 30 March 2016.
Accepted: 22 August 2017.
Published online first: 8 September 2017.
https://doi.org/10.21141/PJP.2017.011

Corresponding author: Francis F. Dematera, MD
E-mail: francisdematera@yahoo.com

INTRODUCTION

The copper sulphate method for hemoglobin screening in blood donation has been in use for decades. The Far Eastern University-Nicanor Reyes Medical Foundation (FEU-NRMF) Medical Center, a tertiary hospital, previously utilized a colorimetric method using an automated analyzer for walk-in and mobile blood donation for potential blood donors until 2010, when it shifted to copper sulphate method to alleviate the cost of hemoglobin screening and the total cost of blood donor screening. The method is not only considered to be the most economical in FEU-NRMF and in the Philippines, but also in other developing countries.[1]

The copper sulphate method determines the hemoglobin content in a drop of blood by inference from the specific gravity of the copper sulphate solution. A drop of blood upon contact with the solution becomes encased in a sac of copper proteinate. The dispersion of the drop of blood and any immediate change in the specific gravity of the solution due to blood's dispersion is prevented for about 15 seconds.[2] The minimum hemoglobin requirement for blood donation is 12.5 g/dl and with a copper sulphate solution with specific gravity of 1.053, a drop of blood with hemoglobin of 12.5 g/dl or higher should sink in 10 to 15 seconds while a drop of blood with hemoglobin level of lower than 12.5 g/dl the drop of blood should float.[3]

METHODOLOGY

An experimental study was conducted by doing experiments of adding another 25 drops of blood to a 100-ml copper sulphate solutions that have been used for 25 tests, one drop of blood per test, using samples of known hemoglobin levels at assigned working temperatures.

Subjects were chosen by judgment sampling. Individuals who were invited to participate in the study were blood donors who had undergone hemoglobin screening utilizing microcuvette technology (Hemocue) using capillary blood, and hospital employees with a recent complete blood count with low hemoglobin level. Possible subjects were given the option to participate or not to participate in the study. The details of the study were explained to them and they were given consent forms. Those who accomplished the consent forms were included in the study. Thirty subjects were chosen. Blood samples were extracted from the subjects and hemoglobin level of each sample was determined using a hemoglobin analyzer employing photometry as principle. Blood samples were grouped based on hemoglobin level, ten samples having hemoglobin level of less than 12 g/dl as one group, ten samples with 12 to 14 g/dl and remaining ten as one group with more than 14 g/dl.

The experiments in the study were conducted in the following order:

1. Blood collection from subjects by a medical technologist
2. Hemoglobin level determination of blood samples
3. Grouping of blood samples as to three hemoglobin ranges
4. Testing
5. Recording of results

The copper sulphate solution used in the experiments was prepared by a medical technologist following the standard operating procedure of FEU-NRMF Medical Center which also conforms with the procedure stated in the USAID-published Anemia Detection Methods: A Manual for Health Workers. Please see appendix B and C for the preparation of the copper sulphate solution. The measurement of specific gravity of copper sulphate solution throughout the conduct of the study was performed by a medical technologist who did not perform any other part of the experiment.

Blood samples were collected by a medical technologist by venipuncture from each of the thirty subjects and transferred to tubes with spray-dried EDTA anticoagulant. Hemoglobin level of each blood sample was determined using a hemoglobin analyzer employing photometry as principle. Samples were grouped as to the three hemoglobin ranges, ten samples per group. Samples with hemoglobin level of less than 12 g/dl were assigned in group A, samples with hemoglobin level of 12 to 14 g/dl in group B and samples with hemoglobin level of more than 14 g/dl in group C. Each sample in the group was assigned with an accession number.

Six experiments were done. Experiments 1, 2 and 3 made use of samples from groups A, B and C, respectively and were conducted at room temperature. Experiments 4, 5 and 6 also made use of the same samples from groups A, B, and C, respectively, but were conducted at working temperature of 29 °C to 30 °C.

Each experiment was performed using 100 mL copper sulphate solution in a glassware with the buoy of the hydrometer that gave the solution a depth of 3 inches. One drop of blood was one test. Blood samples in the group were rotated to complete the 50 tests. As in the usual use of copper sulphate solution for hemoglobin screening, the drops of blood in the solution were observed for 15 seconds if they would sink or float. The specific gravity was 1.053 at the start of each experiment. Twenty-five tests were performed successively with no change in specific gravity of the solution measured after the twenty-fifth test. To complete the fifty tests, another 25 tests were performed and the specific gravity was determined after every 5 tests. The measurement of specific gravity was conducted by lifting the buoy of the hydrometer above the solution and lowering it into the solution with a light spinning motion. Once the buoy has stopped moving, the plane of the surface of the solution that intersected the scale on the stem of the buoy was taken as the specific gravity. Measurement of the specific gravity was done without any drop of blood touching the buoy of the hydrometer. The specific gravity in each measurement was recorded to determine if there would be change.

The investigator did not use any statistical test as the data could be analyzed without calculation. The measured specific gravity at the start of experiment, the specific gravity after the first twenty-five tests and every additional five tests to complete fifty tests were tabulated per experiment. Experiments 1, 2, and 3 are presented in Table 1, and experiments 4, 5, and 6 in Table 2.

Table 1. Specific gravity per number of test and hemoglobin level at room temperature in experiments 1, 2 and 3

Number of Test (Drops of Blood)	Specific Gravity of 100 mL Copper Sulphate Solution		
	Experiment 1 (<12 g/dL Hemoglobin Level)	Experiment 2 (12 to 14 g/dL Hemoglobin Level)	Experiment 3 (>14 g/dL Hemoglobin Level)
0	1.053	1.053	1.053
25	1.053	1.053	1.053
30	1.053	1.053	1.053
35	1.053	1.053	1.053
40	1.053	1.053	1.053
45	1.053	1.053	1.053
50	1.053	1.053	1.053

Table 2. Specific gravity per number of test and hemoglobin level at temperature 29-30°C in experiments 4, 5 and 6

Number of Test (Drops of Blood)	Specific Gravity of 100 mL Copper Sulphate Solution		
	Experiment 1 (<12 g/dL Hemoglobin Level)	Experiment 2 (12 to 14 g/dL Hemoglobin Level)	Experiment 3 (>14 g/dL Hemoglobin Level)
0	1.053	1.053	1.053
25	1.053	1.053	1.053
30	1.053	1.053	1.053
35	1.053	1.053	1.053
40	1.053	1.053	1.053
45	1.053	1.053	1.053
50	1.053	1.053	1.053

The change in specific gravity as to the number of tests was determined by observing for any increase in the specific gravity during performance of additional 25 tests in all six experiments.

The change in specific gravity as to hemoglobin level of the samples was determined by making a comparison of the measured specific gravity between experiments that used samples from groups with different hemoglobin ranges. Experiments 1 and 2, 2 and 3, 1 and 3, 4 and 5, 5 and 6, and 4 and 6 were compared.

The change in specific gravity as to temperature was determined by comparing the measured specific gravity between experiments

1 and 4, 2 and 5, and 3 and 6 which used samples belonging to same hemoglobin range but were conducted at different working temperatures.

RESULTS

There was no change in the specific gravity of 100 mL copper sulphate solution used for fifty tests in the six experiments (Tables 1 and 2). There was no difference in the measured specific gravity between experiments that were compared.

DISCUSSION

To maintain the sensitivity of the test, the United Kingdom Blood Transfusion and Tissue Transplantation Service, the World Health Organization in its publication entitled Model Standard Operating Procedure for Blood Transfusion Service and the American Association of Blood Banks recommend changing the solution after it has been used for 25 tests because the solution is expected to give unreliable results thereafter.[3,4,5] However, in the Anemia Detection Methods for Low Resource-Settings: A Manual for Health Workers published by USAID in 1997, the copper sulphate solution with the same specific gravity used for male patients in anemia screening recommends that it can be used for 50 tests.[6] The effect on the reliability of the test was not specified in the above stated publications if it is due to change in specific gravity secondary to the effect of temperature to the solution, the effect of hemoglobin content of the blood being tested or performance of more than 25 tests with one drop of blood per test. Although the solution changes in color after performance of several examinations, performance of 50 tests can be done in a 100-ml copper sulphate solution with no limitation in the visualization of blood dropped in the solution.

Phillips et al., in their article that introduced the copper sulphate method for hemoglobin screening, mentioned that correction for temperature is not needed during the testing as the coefficient of expansion of copper sulphate solution approximate closely those of blood and plasma but reports have been received by California Blood Bank Society that the specific gravity of copper sulphate solution used in screening hemoglobin for potential blood donors change with temperature.[5]

The copper sulphate method of estimating hemoglobin has been thought by many to be an obsolete test due to the advent of new technologies. But due to the simplicity of the procedure and lower cost, the technique is still widely used even in the United Kingdom.[3] In the Philippines, the Philippine Blood Center still uses copper sulphate in donor screening for hemoglobin. Sawant et al., showed that 29% of potential donors who were deferred had hemoglobin level of more than 12.5 g/dl in a study involving 400 blood donors.[7] However, in the prospective study evaluating the quality and the cost of four hemoglobin screening methods, automated analyzer being the standard, done by Tondon et al., in 2009, they found out that the copper sulphate method still stands the test of time and remains the primary screening method.[1] They, however, recommended that to avoid the inappropriate deferrals, subsequent testing with Hemocue, a hemoglobinometer employing the photometer principle, should be done.[1]

The American Association of Blood Banks or AABB recommends 12.5 g/dl as the minimal hemoglobin requirement for an allogenic donor.[8] The FEU-NRMF Medical Center follows the recommendation of AABB for the minimal hemoglobin requirement for blood donation. In other countries, such as the United Kingdom, there are separate criteria for male and female donors, 12.5 g/dl for the former and 13.5 g/dl for the latter,[3] a recommendation also made by the United States Agency for International Development (USAID). AABB recommends the use of at least 30 mL working solution that will allow a drop of blood to fall approximately 3 inches after dropping (about 1 cm above the surface of the solution).[8] The USAID publication states that 100 mL of working solution should be used with no mention of the length that a drop should fall from the upper surface of the solution to the inner surface of the bottom of the container, with no recommended height of the fall of the drop of blood from the capillet to the surface of the solution.[1] At the FEU-NRMF Medical Center, a 100 mL working solution in a 100 mL beaker is used for testing, giving the solution an approximate depth of 3 inches from the upper surface of the solution to the bottom of the container. The blood is dropped 1 cm above the surface of the copper sulphate solution.

The difference between the practice in FEU-NRMF Medical Center and the recommended standard operating procedures of the USAID (with the exception of the separate hemoglobin cut-off for non-pregnant females, the height of the fall of the drop of blood from the capillet to surface area of solution, and the depth that a drop of blood must sink from the surface of the solution to the bottom), is the number of drops of blood that can be tested in a 100 ml copper sulphate solution with specific gravity of 1.053. The standard operating procedure of FEU-NRMF Medical Center is to change the solution after 25 drops have been introduced which conforms with the Model Standard Operating Procedures for Blood Transfusion Service published by the World Health Organization and the Technical Manual of American Association of Blood Banks.[3,4,8] The USAID, however, has a different recommendation of using a 100 ml copper sulphate solution for 50 tests, which if proven to still maintain its specific gravity and therefore its sensitivity with the addition of another 25 tests in the same solution, will be of great benefit to blood banks that still use the method.[6]

Apart from the hemoglobin level of the blood, the working temperature and other proteins may interfere with the testing. Although it is said that correction for temperature is not needed because the coefficient of expansion of copper sulphate solution approximate closely those of blood and plasma.[5] Reports have been received by the California Blood Bank Society in its e-Network Forum about the change in specific gravity with temperature.[2] Other proteins may also interfere and the outcome may be deleterious if an anemic donor will be bled. Such is the case of a patient who was bled with only 8 g/dl of hemoglobin, the patient passed the copper sulphate method due to hyperproteinemia secondary to multiple myeloma.[9] These other proteins in serum or whole blood may be tested using the same principle with copper sulphate method which means that they can influence the result of hemoglobin screening.[10] This is one of the limitations of Copper Sulphate method in hemoglobin screening. However, such a case has not yet been reported in FEU-NRMF Medical Center.

Two confounding variables were identified. One was the effect of the use of venous blood with liquid anticoagulant and not capillary blood. Since no study was found stating the difference between the weight of anticoagulated blood and blood without

anticoagulant, it was assumed that the anticoagulant present in the blood collection tubes could affect the result of the experiment by diluting the blood. To minimize the possible error associated with the use of liquid anticoagulant, spray dried ethylene diamine tetraacetic acid-anticoagulated (EDTA) lavender top tubes were used. During the conduct of the study, another confounding variable that was identified was the adherence of blood to the stem of the buoy of hydrometer, adding to the weight of the buoy, which caused a false decrease of the specific gravity. The plane of the surface of copper sulphate solution will intersect with a lower specific gravity number as the buoy becomes heavier. To resolve the problem, the other method of measuring specific gravity using refractometer was entertained, but was not employed as the method requires removal of drops of copper sulphate solution for the tests, decreasing the volume of the solution.

One limitation of the study is the conduct of experiments in only two working temperatures, room temperature, which is the recommended working temperature in blood banks and laboratories, and at a higher temperature of at least 29°C. Therefore, the study cannot make a recommendation for working temperature lower than that of room temperature.

CONCLUSION

This paper has shown that there is no change in the specific gravity of a 100 ml copper sulphate solution used for fifty tests with sample of various hemoglobin levels conducted at different working temperatures. Thus, a 100-mL copper sulphate solution can be used for fifty tests using samples of various hemoglobin levels at room temperature and at a higher temperature.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Tondon R, Verma A, Pandey P, and Chaudhary R. Quality evaluation of four hemoglobin screening methods in a blood donor setting along with their comparative cost analysis in an Indian scenario. Asian J Transfus Sci. 2009;3(2):66-9. https://doi.org/10.4103/0973-6247.53874.
2. Problems with QC of Copper Sulfate? E-Network Forum-California Blood Bank Society.
3. Guidelines for blood transfusion services in the United Kingdom, 7th ed., UK blood transfusion and tissue transplantation services, 2005. Available online: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/228828/0117033715.pdf.
4. Model standard operating procedures for blood transfusion service. World Health Organization, New Delhi, 2002. Available online: http://apps.searo.who.int/PDS_DOCS/B0235.pdf?ua=1.
5. Philips RA, Van Slyke DD, Hamilton PB, Dole VP, Emerson K, Archibald RM. Measurement of specific gravities of whole blood and plasma by standard copper sulfate solution. J Biol Chem. 1950;183:305-30. Available online: http://www.jbc.org/content/183/1/305.full.pdf+html.
6. Anemia detection methods in low-resource settings: a manual for health workers. Seattle, Washington, USA: PATH Publications, 1997. Available online: http://www.path.org/publications/files/TS_anemia_guide_health_workers.pdf.
7. Sawant RB, Bharucha ZS, Rajadhyaksha SB.Evaluation of hemoglobin of blood donors deferred by copper sulfate method for hemoglobin screening. Transfus Apher Sci. 2007;36(2):143-8. PMID: 17382593. https://doi.org/10.1016/j.transci.2006.11.001.
8. Brecher ME, American Association of Blood Banks. Technical Manual, 15th ed. Bethesda, Md.: American Association of Blood banks, 2005. Available online: http://www.eqas.ir/pdf/lib/AABB%20Technical%20Manual%2015TH.pdf.
9. Mannarino AD, Macpherson CR. Copper sulfate screening of blood donors: report of a donor passing the test with less than eight grams of hemoglobin. Transfusion:1963;3:398-400. PMID: 14077075.
10. McPherson RA, Pincus MR, Henry JB. Henry's clinical diagnosis and management by laboratory methods, 21st ed., Philadelphia: Saunders Elsevier, 2007. NLIM ID: 101289889 [Book].

Proficiency Testing of Clinical Laboratories for Bacteriology in the Philippines, 2009–2015

Melisa Mondoy,[1] Julius Matt Rapanut,[1] Mark Philip Bugayong,[1] Razaele Aguinaldo,[1] Rafael Navarro,[1] Kristine Jeanne Yap,[1] Ma. Theresa Kapawan,[1] Daryl Joy Almonia,[1] Grace Esparar,[1] Alexander Sadiasa,[1] Noel Macalalad,[2] Lydia Sombrero,[1] Maria Rosario Capeding,[1] Socorro Lupisan[3]

[1]*Department of Microbiology, Research Institute for Tropical Medicine – Department of Health, Philippines*
[2]*National Tuberculosis Reference Laboratory, Research Institute for Tropical Medicine – Department of Health, Philippines*
[3]*Office of the Director, Research Institute for Tropical Medicine – Department of Health, Philippines*

ABSTRACT

Introduction. The National External Quality Assessment Scheme (NEQAS) has been established by the Department of Health–Philippines (DOH) to provide DOH-approved external quality assessment programs, including the Proficiency Test (PT) for Bacteriology to clinical laboratories. The PT for Bacteriology aims to monitor and evaluate laboratory capabilities in the identification of clinically important pathogens through proficiency testing. Since then, participation in the NEQAS has been a requirement for clinical laboratories to obtain a license to operate from the DOH–Health Facilities and Services Regulatory Bureau (HFSRB).

Objective. The objective of this report is to summarize and examine the results of the PT for Bacteriology from 2009 to 2015 and the performances of participating clinical laboratories throughout the Philippines.

Methodology. The Research Institute for Tropical Medicine National Reference Laboratory (RITM-NRL) conducted orientation seminars between 2008 and 2009 to introduce clinical laboratories to the NEQAS. Laboratories submitted their accomplished enrolment forms to RITM–NRL and paid the fees to enroll in the PT. Participating laboratories were required to identify three analytes and perform antimicrobial susceptibility test (AST) on one assigned analyte.

Results. A total of 468 laboratories participated over the seven-year period. The number of participating laboratories obtaining a passing score of 80% and above had significantly increased from 2009 to 2015. Out of the 144 laboratories consistently enrolled over the seven-year period, the proportion of participants with scores of 80% and above had increased. Of the 468 participating laboratories throughout 2009 to 2015, 33.3% were good performers; 6.6% were fair performers; and 60.0% were poor performers.

Conclusion. The increasing number of participating laboratories obtaining passing scores over the years suggests overall improvement of the performance of clinical laboratories in bacteriology. Corrective actions are still needed to address the situation regarding the poor performing laboratories. The assessments done in 2008 and 2013 found that poorly performing laboratories lack trained personnel, resources, and implementation of quality assurance procedures for bacteriological testing.

Key words: laboratory proficiency testing, bacterial identification, antibiotic susceptibility testing

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 29 September 2017.
Accepted: 2 November 2017.
Published online first: 3 November 2017.
https://doi.org/10.21141/PJP.2017.012

Corresponding author: Melisa U. Mondoy, RMT, MPH
E-mail: mum_2468@yahoo.com

INTRODUCTION

Clinical bacteriology laboratories carry out the detection and isolation of bacterial pathogens for management and surveillance of infectious diseases. They are responsible for detecting antibiotic resistance, identifying outbreak pathogens, and communicating incidences and information concerning infectious diseases to public health authorities. These important tasks are the reason that high quality testing, and accurate and precise results must always be ensured.[1]

The Proficiency Test (PT) for Bacteriology assesses the ability of clinical microbiology laboratories to identify and characterize clinically important bacteria and conduct antimicrobial susceptibility tests. It aims to improve the performances of laboratories and ensure high quality and reliable testing in the field of clinical bacteriology.[2,3] In other countries, external quality assessment schemes (EQAS) and PTs for clinical microbiology,

had been influencing the improvement of the quality of testing and performance of participating laboratories in different time periods.[4–7] Improvement of laboratory performance can be attained when sources of errors that result to poor performance are identified and addressed.[4,8,9]

In order to establish an effective public health laboratory network in the Philippines and streamline its functions, the Department of Health (DOH) of the Republic of the Philippines issued the Department Order No. 393–E s. 2000. It designated the Department of Microbiology of the Research Institute for Tropical Medicine (RITM) as the National Reference Laboratory (NRL) for bacterial enteric diseases, emerging and re-emerging bacterial diseases, and mycology. It also mandates RITM–NRL to maintain a quality assurance program for clinical bacteriology laboratory tests.[10] The Department Administrative Order No. 2007–0027 and Memorandum No. 2009–0086, were then issued by the DOH, which required every clinical laboratory throughout the country to participate in the National External Quality Assessment Schemes (NEQAS) in order to obtain a license to operate (LTO) from the DOH Health Facilities and Services Regulatory Bureau (HFSRB, formerly Bureau of Health Facilities and Services).[11,12] NEQAS issues DOH-approved external quality assessment programs for bacteriology, parasitology, and mycobacteriology to clinical laboratories by providing a proficiency test that aims to monitor and evaluate the laboratory's capabilities to identify clinically important pathogens. Thus, the RITM–NRL has been providing annual PTs for bacteriology under the NEQAS for Bacteriology, Parasitology, and Mycobacteriology to clinical laboratories since 2009.

This report summarizes and examines the results of the PT for Bacteriology from 2009 to 2015 and the performances of participating clinical laboratories. The information and analysis were only limited to the scores, regional location, ownership type and accreditation category of 468 participants in the PT throughout the seven-year period and to the data acquired from the assessments of selected participating laboratories.

METHODOLOGY

Baseline assessment of laboratories

A baseline on-site assessment of tertiary clinical laboratories throughout the country was conducted by RITM-NRL in 2008. The assessment aimed to (1) monitor their compliance with the minimum and essential requirements as set by RITM–NRL (Table 1); (2) evaluate their capacity to isolate, identify, and characterize medically important bacterial pathogens; (3) identify their deficiencies, which may result to poor performance in bacteriological testing; (4) educate laboratorians on current microbiological advancements; (5) and promote good laboratory practices. The list of tertiary laboratories provided by HFSRB served as the basis for the number of laboratories to be assessed. The assessment covered laboratory practices on specimen processing: (1) isolation and identification of medically important bacteria; (2) methods for antimicrobial susceptibility testing; (3) internal and external quality assessment practices; (4) use of functional equipment, instruments, culture media, reagents, kits, glassware, and disposables; and (5) waste management.

Table 1. Number of times participants passed vs number of PT participation, 2009-2015

No. of Annual Participation	Number of Times Passed								Total
	0	1	2	3	4	5	6	7	
1	33	16							49
2	21	4	3						28
3	19	22	8	1					50
4	8	10	14	8	1				41
5	13	13	11	13	6	3			59
6	16	14	18	13	16	5	3		85
7	8	21	29	25	30	20	14	9	156
Total									468

Legend: Poor Fair Good

Table 2. Biological standards used to perform quality control on the analytes and frequency of incorrect ID 2009-2015

Analyte (Binomial Name and Authority)	ATCC® Standard	No. of ID	Incorrect ID (Percentage)
Acinetobacter lwoffii (Audureau) Brisou	17925	27	14 (51.9%)
Moraxella catarrhalis (Frosch and Kolle) Bovre	25238	544	278 (51.1%)
Candida tropicalis (Castellani) Berkhout	10610	10	5 (50.0%)
Enterobacter aerogenes Hormaeche & Edwards	13048	95	43 (45.3%)
Haemophilus influenzae (Lehmann & Neumann) Winslow et al.	49247	944	418 (44.3%)
Streptococcus pneumoniae (Klein) Chester	49619	766	317 (41.4%)
Enterococcus faecalis (Andrewes & Horder) Schleifer & Kilpper-Balz	19433	444	170 (38.3%)
Morganella morganii (Winslow et al.) Fulton	25830	352	131 (37.2%)
Streptococcus pyogenes Rosenbach	12344	89	32 (36.0%)
Streptococcus agalactiae Lehmann & Neumann	13813	378	134 (35.5%)
Shigella flexneri Castellani & Chalmers	12661	389	128 (32.9%)
Klebsiella oxytoca (Flugge) Lautrop	13182	748	233 (31.1%)
Klebsiella pneumoniae subsp. *pneumoniae* (Schroeter) Trevisan	13883	36	11 (30.6%)
Salmonella enterica subsp. *enterica* sv. Enteritidis[1] (ex Kauffmann & Edwards) Le Minor & Popoff	49223	117	34 (29.1%)
Vibrio cholerae Pacini	14035	74	21 (28.4%)
Enterobacter cloacae subsp. *cloacae* (Jordan) Hormaeche & Edwards	35929	58	14 (24.1%)
Staphylococcus epidermidis (Winslow & Winslow) Evans	12228	148	34 (23.0%)
Serratia marcescens Bizio	43862	70	16 (22.9%)
Staphylococcus saprophyticus (Fairbrother) Shaw et al.	49453	505	113 (22.4%)
Proteus mirabilis Hauser	29906	101	20 (19.9%)
Pseudomonas aeruginosa (Schroeter) Migula	27853	114	16 (14.0%)
Salmonella enterica subsp. *enterica* sv. Typhi[2] (ex Kauffmann & Edwards) Le Minor & Popoff	19430	333	40 (12.0%)
Candida albicans (Robin) Berkhout	90028	506	60 (11.9%)
Staphylococcus aureus subsp. *aureus* Rosenbach, methicillin-resistant[3]	BAA-1720	451	50 (11.1%)
Staphylococcus aureus subsp. *aureus* Rosenbach	25923	112	10 (8.9%)
Escherichia coli (Migula) Castellani & Chalmers	25922	55	3 (5.5%)
	Total	7,466	2,345 (31.4%)

[1] Classified as non-typhoidal *Salmonella* (NTS) along with other serovars such as Typhimurium
[2] Also referred to as *Salmonella* Typhi
[3] Also known as MRSA

Enrolment in the proficiency tests

Since the DOH required clinical laboratories registered under tertiary category in the Center for Health Development (CHD) to participate in the PT event for the first time in 2009, RITM–NRL conducted orientation seminars between 2008 and 2009 to introduce the participants to the NEQAS. Participating laboratories submitted their accomplished enrolment forms through courier, fax, e-mail, or personal delivery and paid the fees before the scheduled testing event to be eligible for the PT. In 2015, DOH required all clinical laboratories under primary, secondary, and tertiary category performing bacteriological testing to participate in the PT.[13]

Materials and analyte preparation

Analyte culture, inoculation, and verification

Clinically-significant bacteria were subcultured from stock cultures in skim milk-tryptone-glucose-glycerol (STGG) media[14] incubated at 36±1°C for 18 to 24 hours. Cultures were examined for purity; assayed through conventional identification methods[15] and commercial identification testing systems: API® (bioMérieux, Marcy-l'Étoile, France) and VITEK® 2 (bioMérieux, Marcy-l'Étoile, France); and compared to ATCC® biological standards (ATCC, Manassas, Virginia, USA) to verify their identities (Table 2). The analytes were inoculated in semi-solid sheep blood agar (SBA)[16] contained in 2 mL-cryogenic vials (Corning Inc., Corning, New York, USA) and incubated at 36±1°C for 18 to 24 hours prior to transport.

Verification of analyte antimicrobial susceptibility

Antimicrobial susceptibility of analytes was verified using BBL™ Sensi-Disc™ Susceptibility Test Discs (Becton, Dickinson, and Co., Sparks, Maryland, USA). The panel of antibiotics used was based on the Performance Standards for Antimicrobial Susceptibility Testing of the Clinical and Laboratory Standards Institute (CLSI, Wayne, Pennsylvania, USA).[17]

Packaging

Analytes sent to participating laboratories were packaged in accordance to the international standard of transporting biohazard materials.[18,19] Each vial was sealed with Parafilm M® (Bemis Co. Inc., Oshkosh, Wisconsin, USA), individually wrapped in a paper towel, and placed inside a 100 mm × 150 mm resealable polypropylene resin bags along with other analytes. The wrapped vials were then encased in 600 mL polypropylene canister (Philtop Industries Inc., Valenzuela City, Metro Manila, Philippines) and placed inside a 120 mm × 115 mm × 190 mm corrugated box (Thousand Oaks Packaging Corp., Parañaque City, Metro Manila, Philippines) with the necessary attachments and labels. The package also includes standard proficiency testing guidelines that contain basic information and instructions needed for the handling of the analytes and an answer sheet.

Quality Control of Transport Media and Packaged Analytes

The semi-solid sheep blood agar (SSBA) to be used as transport media was assured for sterility and tested for culture response before use in the PT. Four sets of cultures of each of all the organisms used as analytes in the PT were prepared and each set was subjected to each of the four different treatments. For the first treatment, separate packages containing one set of different analytes were sent to random locations of participating laboratories throughout the country. The selected laboratories were asked to return the sealed package to RITM. Upon return, the analytes were examined for contamination by unwanted organisms and tested for viability through routine culture examination. For the remaining treatments, three sets were incubated at 36±1°C, 4±2 °C, and ambient temperature, respectively. Growth was observed after three, five, and seven days. After seven days of incubation, the analytes were subcultured and re-identified through conventional methods and commercial identification systems such as API® and VITEK® 2.

The Bacteriology PT program

Participating laboratories were asked to identify each of the three analytes by means of their routine methods or standard operating procedures. They were also required to perform antimicrobial susceptibility testing on one pre-assigned analyte using the panel of antibiotics recommended by CLSI. After receiving the analytes, participants were given fifteen working days to complete the PT.

The Overall Score, with a perfect rating of 100%, comprised of 75% for organism identification and 25% for AST. RITM–NRL set the passing score to be 80%. A correct identification—with correct binomial name—amounts to 25 points; an acceptable identification—with correct genus but incorrect or unspecified species—amounts to 10 points; an incorrect one amounts to no point. A correct report of antibiotic susceptibility amounts to one point while an incorrect report amounts to no point. The identification of an additional organism would result into an addition to the number of principal organisms in the equation but no addition to the points corresponding to the correct and acceptable identification. This would eventually lead to the reduction of the overall score. The following equation represents the computation for the overall score:

$$\text{Overall Score (\%)} = \frac{\left(\begin{matrix}\text{no. of}\\ \text{correct}\\ \text{ID}\end{matrix}\right)\times 25 + \left(\begin{matrix}\text{no. of}\\ \text{acceptable}\\ \text{ID}\end{matrix}\right)\times 10}{\left(\begin{matrix}\text{no. of}\\ \text{principal}\\ \text{organisms}\end{matrix}\right)\times 25}\times 75\% + \frac{\begin{matrix}\text{no. of}\\ \text{correct AST}\\ \text{report}\end{matrix}}{\begin{matrix}\text{no. of}\\ \text{antibiotics}\end{matrix}}\times 25\%$$

Each of the participating laboratories who had accomplished the PT and submitted their answers to NEQAS received a certificate of participation, a summary of results of its PT performance, and a learning monograph, which recommends standard methods of identifying the analytes and performing AST.

Evaluation of the overall performance of laboratories

Participating laboratories were grouped according to the number of times they enrolled in the annual PTs from 2009 to 2015 and the number of times they got a passing score of 80%. The performances of participating laboratories were classified as "good', "fair", and "poor" based on the number of times they passed the annual PT. "Poor performers" refers to participating laboratories who had not met the 80% passing rate for more than 50% of their annual PT participation. "Fair performers" refers to participating laboratories who had met the passing rate in 50% of their annual PT participation. Finally, "good performers" refers to participating laboratories who had passed more than 50% of their annual PT participation.

Assessment of poor performing laboratories

Participating government laboratories that had been consistently obtaining scores below 80% in the 2009–2012 PTs were selected for an on-site reassessment in 2013. Details on the updated training of laboratory personnel; availability of laboratory equipment, reagents, culture media, antibiotics, and glassware; reliance on automated and/or semi-automated systems for identification

and AST; use of CLSI Performance Standards for Antimicrobial Susceptibility Testing as guide for AST; availability of ATCC® biological standards; and implementation of quality assurance and control programs were identified.

Statistical analysis

Graphs were generated using Matplotlib version 2.0.0 pyplot module[20] in Python and all statistical analyses were done using SciPy version 0.19.0 scipy.stats module.[21] Friedman ranking test was used to detect differences in the annual scores of consistently enrolled participants in 2009–2015 and Nemenyi test was used as the post hoc test to detect differences between the rankings of annual scores obtained from Friedman test. Multiple comparisons were employed using STAC Web Platform version 1.0.[22]

RESULTS AND DISCUSSION

2008 baseline assessment of laboratories

The HFSRB list included 400 tertiary laboratories throughout the Philippines. Three hundred forty-seven (86.8% of 400) tertiary clinical laboratories capable of doing bacteriological testing were assessed: 25.1% (87/347) of which were located in the National Capital Region; 21.6% (75/347) in Mindanao; 21.0% (73/347) in North Luzon; 16.4% (57/347) in South Luzon; and 15.9% (55/347) in the Visayas. Two hundred seventy-five (79.2% of 347) were private; 70 (20.2% of 347) were government-owned; and two (0.6% of 347) were semi-private. The remaining 53 (13.2% of 400) in the list had already undergone closure or downgrade during the assessment.

In the assessment, only 43.8% (152/347) of the laboratories could perform Gram stain, acid-fast stain, negative stain, and wet mount; 17.3% (60/347) implemented internal quality control procedures for media, reagents, stains, and antibiotic disks; and only 3.2% (11/347) used ATCC biological standards for quality control (Table 1). Only 35.7% (124/347) completed the required essential major equipment and instruments; 5.8% (20/347) as to culture media and supplements for primary isolation; 19.3% (67/347) as to media for biochemical tests; 3.3% (11/347) as to supplements for growth and identification. Notably, 67.7% (235/347) of the laboratories were using human blood in the preparation of blood agar plates, instead of the recommended sheep blood23, which was used by 23.6% (82/347) of the laboratories, or horse blood, which was used by the remaining 6.6% (23/347). Only 17.9% (62/347) had complete sugars for carbohydrate utilization tests and 2.9% (10/347) had complete antibiotics for AST. All in all, only 2.6% (9/347) had complete required essential media and reagents.

Of the 97.4% (338/347) remaining laboratories, 57.9% (201/347) used commercially prepared kits while 24.8% (86/347) used automated systems for identification of bacterial pathogens. For AST, 2.9% (10/347) had complete required essential antibiotics while 15.9% (55/347) of the remaining uses automated systems.

Only 82.9% (288/347) of laboratories were capable of performing AST. Moreover, 76.9% (267/347) of laboratories were performing disk diffusion and 16.7% (58/347) were using automated systems for AST. Only 47.2% (164/347) were using the latest edition of the Performance Standards for Antimicrobial Susceptibility Testing17 by the Clinical and Laboratory Standards Institute (CLSI), the recommended standard for AST methods and interpretation.

Laboratory participation and performance in 2009–2015

Four hundred sixty-eight participants, comprised of 381 (81.4%) private laboratories and 87 (18.6%) government-owned laboratories, enrolled in the NEQAS for Bacteriology between 2009 and 2015. Of the 450 (96.2% of 468) tertiary category clinical laboratories, 80.7% (363/450) were privately owned, and 19.3% (87/450) were government owned. Eighteen (3.8% of 468) private secondary category laboratories also enrolled in the PTs. The number of participants was highest in the National Capital Region (Figure 1) with 78 (27.3% of 286) participants in 2009, which grew to 112 (29.5% of 403) in 2015.

The annual number of participants were the following: 286 (2009), 285 (2010), 264 (2011), 355 (2012), 364 (2013), 360 (2014), and 403 (2015). Scores ranged from 0 to 100 in all years from 2009 to 2015. The mean scores and sample standard deviation per year were: 63.9±28.2 (2009), 70.6±25.0 (2010), 63.1±24.6 (2011), 57.9±28.6 (2012), 60.0±34.3 (2013), 71.1±24.0 (2014), and 75.2±27.3 (2015). The annual median scores were: 71.4 (2009), 75.1 (2010), 68.8 (2011), 60.0 (2012), 72.0 (2013), 72.0

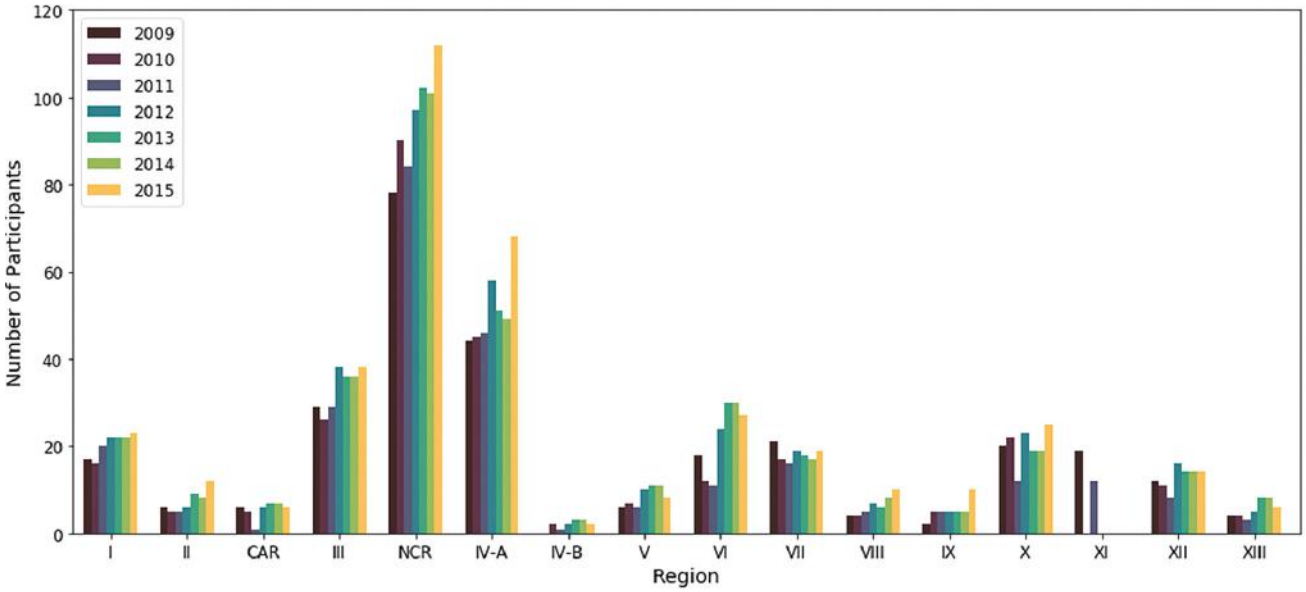


Figure 1. Number of participants from different regions in the Philippines, 2009–2015.

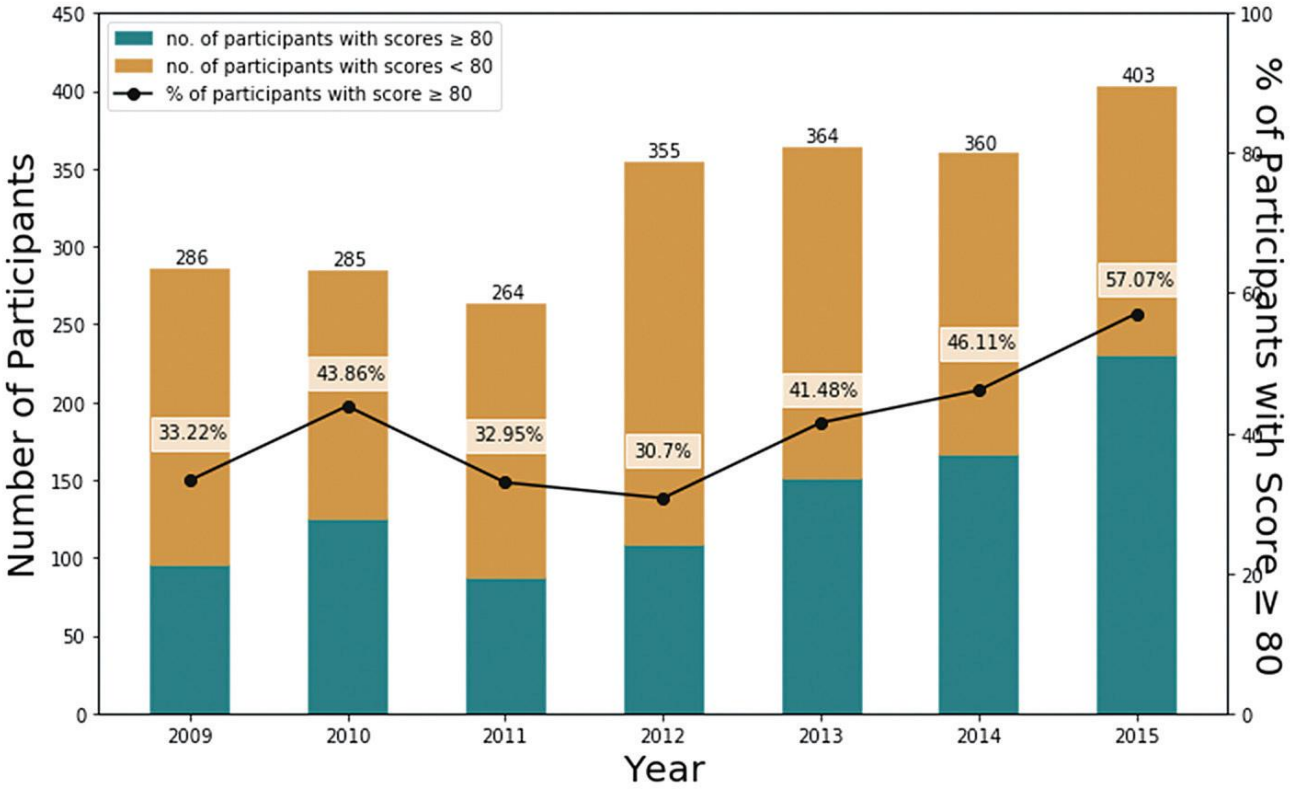


Figure 2. Number of participating laboratories and proportion of passers, 2009–2015; spearman's ρ=0.821, for both annual number of participants and number of passers; $\hat{p}_{2015} - \hat{p}_{2009} = 23.9\%$ and $\chi^2 = 38.13$

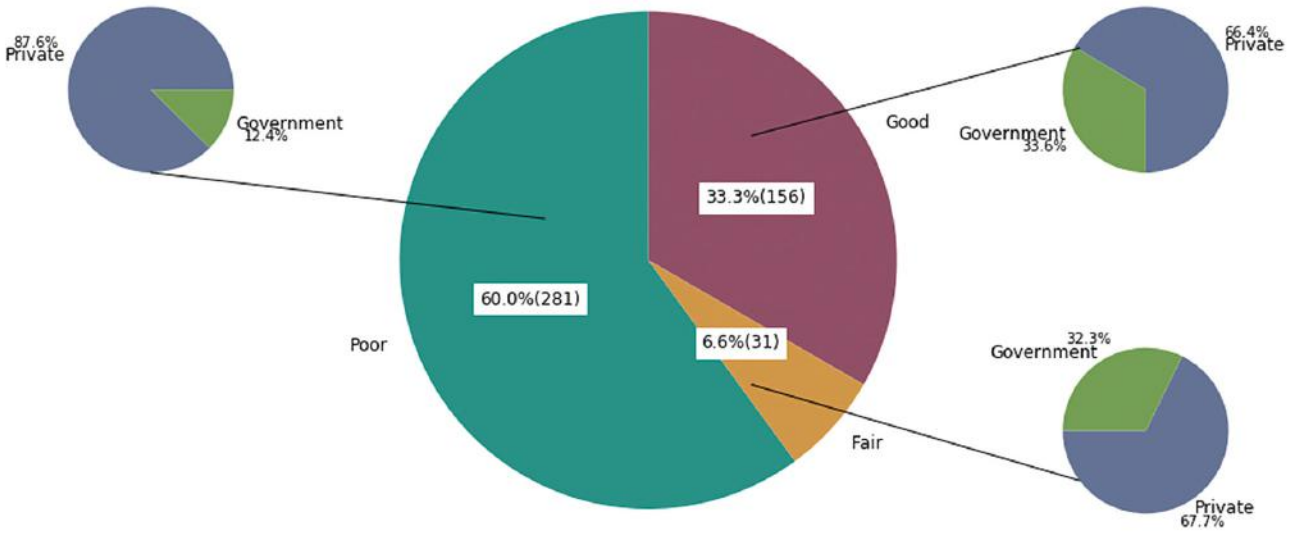


Figure 3. Classification of good, fair, and poor performers, 2009–2015, n=468.

(2014), and 80.0 (2015). The number of participants who obtained scores of 80 and higher increased from 2009 (33.2%, 95/286) to 2015 (57.1%, 230/403). Proportion of passing scores among laboratories participated in PT in bacteriology in 2009–2015 are represented by a line plot (Figure 2).

Out of the 156 (33.3% of 468) good performers, 23.1% (36/156) participants passed all of the annual PTs they enrolled in the span of seven years (Table 1). One hundred eighteen (25.2% of 468) laboratories, on the contrary, never got a passing score in all the PTs, in which they enrolled. Overall, poor performers comprise the most number (60.0%, 281/468) of participating laboratories (Figure 3). A proportion of 40.2% (35/87) government laboratories and 70.4% (247/381) of privately owned laboratories performed poorly in the PT over the seven-year period. While the overall number of participating laboratories and the number of passers were increasing, majority of participating laboratories performed poorly in the PT over the seven years.

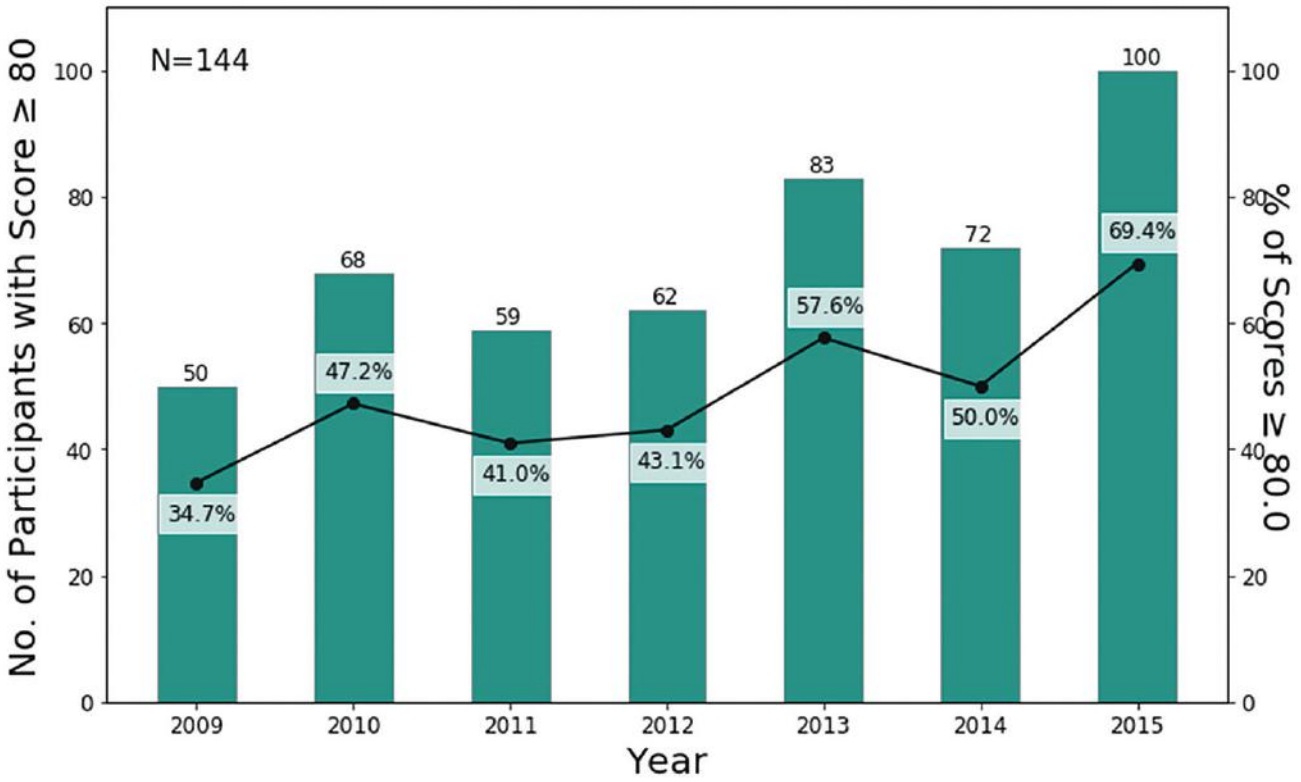


Figure 4. Number and proportion of passers out of laboratories consistently enrolled, 2009–2015; spearman's ρ=0.821 for the annual proportion of passers of consistently enrolled laboratories.

One hundred forty-four laboratories (30.7% of 468) consistently enrolled in the 2009–2015 PTs. The number of laboratories with scores of 80% and above increased from 2009 (37.2%, 50/144) to 2015 (69.4%, 100/144) (Figure 4). Furthermore, the distributions of scores over seven years are negatively skewed, which means the mass of the distribution of scores each year is concentrated in the region of high scores (Figure 5). The majority of distributions of scores in 2010, 2011, 2013, 2014, and 2015 are clustered, resulting to sharper peaks (leptokurtic profiles). The distribution of scores in 2015 holds the highest kurtosis (excess kurtosis = 2.06), which is the measure of the sharpness of the peaks, because of a surge of participants obtaining scores between 90% and 100% (50.7%, 73/144). The highest mean score is found in 2015 ($\bar{x}$ = 81.7) while the lowest mean score is in 2012 ($\bar{x}$ = 67.1), followed by the mean score in 2009 ($\bar{x}$ = 67.6) (Fig. 5). Comparison of annual scores using Friedman test ($F = 10.34$; $P < 0.001$) and Nemenyi test as post hoc analysis shows a significant difference between scores in 2009 and in 2015 ($Z = 5.55$; $P < 0.001$) with 2015 ranking as the highest ($\bar{r}$ = 4.95), 2009 as the third from the lowest ($\bar{r}$ = 3.54), and 2011 as the lowest ($\bar{r}$ = 3.39) in seven years. The increasing proportion of passers in the 144 consistently enrolled laboratories and the significant difference between scores in 2009 and in 2015 suggest that the performance of the consistently enrolled laboratories had generally improved over the seven-year period.

Participation in EQA programs has been proven to improve a laboratory in many ways. It allows the participants to have an idea of their capability and monitor continual improvement, since it generates information that can be used to assess the overall competence and needs of participants.[24] It also brings benefits and challenges to the participants, aside from meeting regulatory requirements.[1] The improvement in the performance of bacteriology laboratories in the Philippines in the Proficiency Test provided by NEQAS is comparable to the improvement of laboratories that participated in different EQAS/PT in other countries. In the 1982–1999 Tokyo Metropolitan Government External Quality Assessment Program, an improvement in the performance of independent laboratories in Tokyo, Japan regarding the identification of H. influenzae, MRSA, and some pathogenic enteric bacteria was observed.[6] In the 1992–1996 Swiss External Quality Assessment Scheme in Bacteriology and Mycology, the increasing mean scores of all participants and the number of participating laboratories with high average scores over the four year period reflected the improving performances of participating laboratories.[7] In the United States, the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) mandate universal requirements for all clinical laboratory-testing sites. This mandate includes the provision of PT that defines laboratory performance. Through PT as one of its tool, CLIA has ensured the adherence of participating laboratories to good clinical practices and improvement in the quality of laboratory tests since 1994.[25] External quality assessment programs are recognized as an effective tool in improving the quality of medical laboratories in Europe.26 Further improvements are being considered to their existing EQA programs such as accreditation of schemes and further integration to information technology[27] that can also be applied in the Philippines.

Quality control of packaged analytes and the analyte material

PTs and EQAS in other countries use either simulated clinical samples or lyophilized cells as analytes. Simulated clinical samples (e.g. nose swabs, artificial feces, simulated spinal fluid, etc.) provide clinically relevant and realistic challenges yet they require appropriate facilities and technology, and arduous effort.28 Lyophilization of cells for transport is straightforward

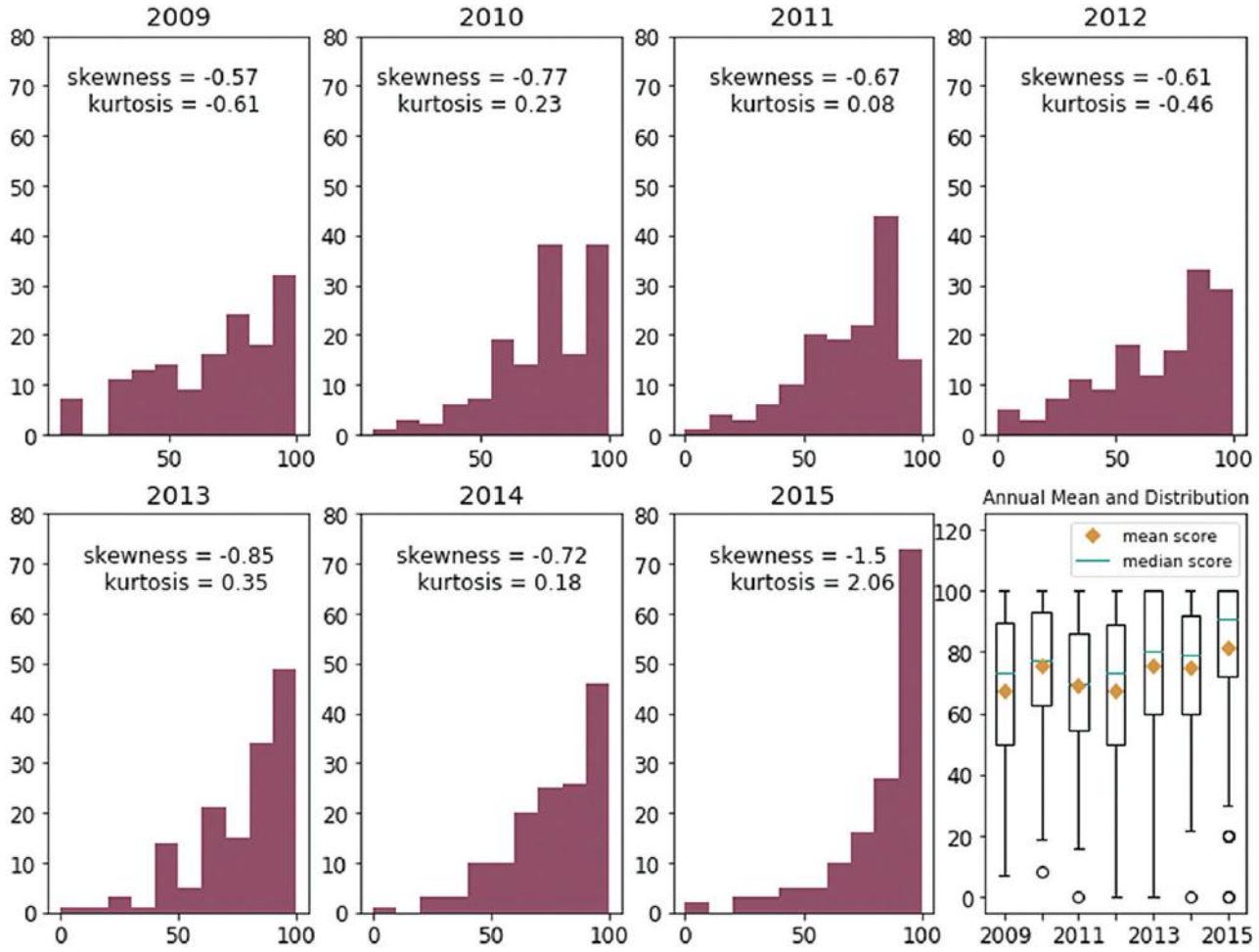


Figure 5. Histogram and box plot of scores plus annual mean scores of 144 laboratories consistently enrolled in the 2009–2015 proficiency tests.

and it protects cells from degradation; however, the subsequent processes of reconstitution and multiple passages do not demonstrate clinical relevance and realism. It completely lacks resemblance to clinical specimens.[4] Furthermore, artificial handling and significant matrix effects can affect the growth, colony morphology, and nutrient metabolism of organisms, thus affecting proper identification and characterization of analytes.[28] In this PT, the use of semi-solid SBA as matrix was proven to be effective in ensuring the viability of organisms during transport, through quality control. All of the isolates sent for quality control throughout 2009 to 2015 were observed to be contaminant-free and viable.

Identification and AST

Bacteriology laboratories in the Philippines used conventional methods, manual commercial kits, and automated systems for the characterization and identification of clinically significant pathogens. The organisms identified with least difficulty were *E. coli*, *S. aureus*, MRSA, *C. albicans*, *S. enterica* sv. Typhi, and *P. aeruginosa*. In contrast, the six organisms identified with highest difficulty were *A. lwoffii*, *C. tropicalis*, *M. catarrhalis*, *E. aerogenes*, *H. influenzae*, and *S. pneumoniae* (Table 2). Additionally, the antibiotic susceptibilities of *S. pneumoniae*, *E. cloacae*, and *H. influenzae* were the most difficult to determine (Table 3).

The organisms identified with least difficulty are usually distinguished with colony examination and few straightforward biochemical tests. *S. aureus* is identified as Gram-positive coccus, which is positive for catalase and coagulase tests. *Salmonella enterica* sv. Typhi can be distinguished from nontyphoidal *Salmonella* with its distinct biochemical characteristics: it is citrate-negative, ornithine-negative, and mucate-negative; it yields alkaline products on aerobic environment, acidic products on anaerobic environment; and it weakly produces hydrogen sulfide gas in triple sugar iron (TSI) agar. Moreover, *S. enterica* sv. Typhi can be differentiated from other Salmonella serotypes through serological tests based on the antigenic properties of the somatic (O:9), flagellar (H-d), and capsular (Vi) antigens. The identity of

Table 3. Frequency of incorrect results per analyte tested for antibiotic susceptibility, 2009-2015

Analyte	Number of AST	Frequency of Incorrect Results (Percentage)
S. pneumoniae	717	337 (47.0%)
E. cloacae	203	87 (42.9%)
H. influenzae	1,422	566 (39.8%)
P. mirabilis	425	149 (35.1%)
S. epidermidis	66	22 (33.3%)
S. enterica (non-typhoidal)	217	61 (28.1%)
P. aeruginosa	424	99 (23.3%)
S. aureus	582	128 (22.0%)
MRSA	2,068	353 (17.1%)
S. flexneri	1,247	190 (15.2%)
S. enterica sv. Typhi	1,585	190 (12.0%)
Total	8,956	2,182 (24.4%)

the yeast, *C. albicans*, can be confirmed when it produces germ tube during germination in horse serum at 37°C, and terminal chlamydospores on hyphae or pseudohyphae during growth in corn meal agar at 25°C. *E. coli* and *P. aeruginosa* can be identified through examination of colony morphology in MacConkey agar and a few basic biochemical tests.[15]

Some of the organisms identified with highest difficulty, *A. lwoffii* and *M. catarrhalis*, are nonfermentative Gram-negative bacteria that belong to the *Moraxellaceae* family. *Acinetobacter* species are oxidase-negative, catalase-positive, indole-negative coccobacillary bacteria. *A. lwoffii* can be differentiated from other *Acinetobacter* species through carbon assimilation tests.[15] *Moraxella* species, on the other hand, are oxidase-positive, catalase-positive, indole-negative coccoid or coccobacillary bacteria. They can be differentiated from the similarly oxidase-positive and catalase-positive *Neisseria* species through examination of the colonies in agar: *Moraxella* colonies may be pushed intact across the plate with a loop like a hockey puck; or through DNase and tributyrin tests. *M. catarrhalis* can be distinguished from other *Moraxella* species through its ability to reduce nitrate and nitrite and its inability to alkalinize acetate and acidify ethylene glycol. *Enterobacter* species belong to the *Enterobacteriaceae* family. *E. aerogenes* can be differentiated from other *Enterobacter* species through lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, and carbohydrate fermentation tests.[15] *C. tropicalis*, a non-*albicans Candida* (NAC) yeast species,[29] is germ tube-negative. It can be distinguished from other *Candida* species through microscopic examination of morphological features of the yeast on cornmeal agar; through carbohydrate assimilation tests; and through carbohydrate fermentation tests.[15]

In the 2008 assessment, majority (66.7%, 231/347) of the laboratories assessed used human blood agar in the isolation, detection, and characterization of clinically important bacterial pathogens. These pathogens, especially fastidious organisms such as *S. pneumoniae* and *H. influenzae*, are less likely to be detected when using human blood agar for culture since antibodies and residual antibiotics in human blood may inhibit the growth of bacterial isolates. It will also result to pathogens producing incorrect or varying hemolysis on the blood plate agar which is one of the characteristics critical when identifying a microorganism. Instead, trypticase soy agar plate with 5% defibrinated sheep, goat, rabbit, or horse blood is recommended for the preparation of blood agar plate and as primary culture plate media for bacterial pathogens.[23] *Streptococcus pneumoniae* and *H. influenzae* are fastidious organisms, which grow best at 36±1°C with around 5%–10% carbon dioxide or in a candle jar. *S. pneumoniae* can be differentiated from other streptococci through characterization of colonies on blood agar plates; optochin test; and bile solubility test using sodium deoxycholate. *H. influenzae*, on the other hand, requires hemin (X factor) and nicotinamide adenine dinucleotide (V factor) for growth; thus, the chocolate agar plate, which contains both factors, is used as the standard growth medium.[15,23] In addition, *S. pneumoniae* and *H. influenzae* must be grown in Mueller-Hinton agar (MHA) supplemented with additional growth factors for AST. MHA with 5% sheep blood is the medium required for AST of *S. pneumoniae* by disk diffusion. Plates must be incubated at 35±2°C with 5% CO2 for 20–24 hours. Haemophilus test medium (HTM; comprised of MHA plus hematin, nicotinamide adenine dinucleotide, and yeast extract) is required for the AST of *H. influenzae* by disk diffusion, and plates must be incubated at 36±1°C with 5%–10% CO2 for 16–18 hours.[17]

Organisms that are difficult to identify require additional tests, equipment, media, supplements, and reagents. Failure to identify organisms that require more complex bacteriological testing can be attributed to the large proportion of laboratories lacking minimum and required essential media and reagents, such as sugars for carbohydrate utilization tests and amino acids for amino acid metabolism tests, based on the result of the 2008 assessment. Also, failure to determine the correct antibiotic susceptibility of bacteria through disk diffusion can be attributed to the large proportion of laboratories that were not using the latest edition of the Performance Standards for Antimicrobial Susceptibility Testing by the CLSI in 2008. Laboratories are expected to correctly interpret the zone diameter breakpoints in disk diffusion according to the updated CLSI standards. Moreover, accurate AST results cannot be achieved with the lack of antibiotics, media (e.g. MHA, etc.), supplements (e.g. non-human mammalian blood, hematin, etc.), and equipment (e.g. CO2 incubators or candle jars, etc.).

2013 Assessment of poor performing government laboratories for bacteriology

Only 31 government-owned laboratories that performed poorly in the 2009–2012 PTs were assessed. Two (6.5% of 31) were found to be non-functional due to a lack of budget. Only 12.9% (4/31) of the laboratories had personnel with updated training; 45.2% (14/31) used ATCC biological standards for quality control; and only 29.0% (9/31) implemented SOPs and quality control of culture media, antibiotics, and equipment. Only 9.7% (3/31) had complete essential equipment; 9.7% (3/31) had complete essential culture media and antibiotics for AST; and none (0/31) of the laboratories had complete essential reagents. On the other hand, 83.9% (26/31) had complete essential glassware. Overall, majority of the assessed laboratories did not meet the minimum and essential requirements, except for having complete essential glassware.

Thirteen (41.9% of 31) laboratories use automated and semi-automated equipment. Six laboratories (19.4% of 31) were using VITEK® 2; four (12.9% of 31) were using API® identification testing kits; and three (9.7% of 31) were using BBL™ Crystal™ Identification Systems (Becton, Dickinson and Co. Diagnostic Systems, Sparks, Maryland, USA). Automated and semi-automated systems require freshly grown isolates within 24 hours as its test template; hence, laboratorians still need the basic materials, equipment, and skills to culture and isolate medically important bacteria and fungi. Laboratorians also need to check the viability and density measurement of the organisms to be tested for AST since automated systems which conducts its AST based on broth microdilution testing method, generally require more than 105 viable cells.[30] Moreover, failure to assign an identification to an organism as a result of low discrimination and discordant identifications by automated and semi-automated systems, still warrants supplemental and confirmatory testing by conventional methods,[31] which require the necessary materials included in the minimum and essential requirements for bacteriological testing.

The baseline assessment of the 347 tertiary laboratories conducted in 2008 and the assessment of 31 poorly performing government laboratories conducted in 2013 found almost similar findings: poor performance was due to poor compliance to the recommended minimum and essential requirements set by RITM–NRL. Both assessments had recommended poor performing laboratories re-training of laboratory personnel; acquisition of the unavailable media, supplements, reagents, and instruments; management on

quality control procedures for media, reagents, antibiotics, and stains; use of the current CLSI standards for AST; and review of skills for bacteria culture, isolation and detection.

More actions are still needed to have a better idea of the current state of clinical bacteriological testing in the country and how it affects the results that are being produced. A wider reassessment, that will aim to include all of the participating laboratories, needs to be carried out urgently and regularly in order to identify factors that lead to poor performance and, likewise, ensure high quality testing and accurate reporting of results. Other information can also be acquired during the reassessment such as compliance or deficiencies in skills, training, resources, and implementation of quality assurance procedures. Further data on the methods used (conventional, commercial or semi-/fully automated system) and how it affects the performance of the laboratory can be investigated. The NEQAS program was established to improve the capacity of participating laboratories in producing quality results. This will lead to the elevation of the state of clinical bacteriology in the country and produce quality service for the Filipino people. In aid of this vision, the RITM together with the DOH offers trainings and assistance that can help the participating laboratories in reaching this goal. Other ideas can be explored to attain this goal such as creating a network among the laboratories that may enable them to share knowledge and resources to improve each other's performance and capabilities. The data that will be gathered in the following years and succeeding plans will be included in future reports and studies.

CONCLUSION

An increasing number of participating laboratories had participated in the PT for Bacteriology and the performances of those consistently enrolled had generally improved over 2009–2015. Moreover, a comparison between distributions of scores over the seven-year period has shown an increase in the number of participating laboratories obtaining high to perfect scores. This progress demonstrates that the NEQAS for Bacteriology had improved the quality and reliability of their methods in identifying bacterial pathogens and detecting antibiotic resistance.

In contrast, the large portion of poorly performing laboratories needs to be addressed. The baseline assessment in 2008 and assessment of poor performers in 2013 identified the deficiencies of clinical microbiology laboratories in skills, training, resources, and implementation of quality assurance procedures. A nationwide reassessment of participating laboratories needs to be carried out urgently and regularly in order to identify factors that lead to poor performance and, likewise, ensure high quality testing and accurate reporting of results.

ACKNOWLEDGMENTS

The authors thank the medical laboratory scientists of the National Reference Laboratories (NRL) under the RITM Department of Microbiology: Josefina Geronimo and Rosa Mate of NRL–Bacterial Enteric Diseases (BED); Rizalina Navarro and Pearl Joy Nazareno of NRL–Mycology; Salvacion Rosario Galit of NRL–Emerging and Re-emerging Bacterial Diseases (ERB); and Gloria Reclusado of NRL–Invasive Bacterial Vaccine Preventable Diseases (IBVPD). The authors likewise thank the following administrative staff: Armando Martinez and Sheila Joy Gonzaga of the RITM–NEQAS for Bacteriology, Parasitology, and Mycobacteriology; and Natalie Del Mundo of the RITM Department of Microbiology.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

The 2009–2012 Proficiency Tests for Bacteriology were funded by the Health Facilities and Services Regulatory Bureau and the Health Facilities Development Bureau—both under the Department of Health of the Republic of the Philippines.

REFERENCES

1. Stang HL, Anderson NL. Use of proficiency testing as a tool to improve quality in microbiology laboratories. Clin Microbiol Newsl. 2013;35(18):145-52. PMCID: PMC4696484. NIHMSID: NIHMS742893. https://doi.org/10.1016/j.clinmicnews.2013.08.007.
2. WHO Southeast Asian Regional Office. Quality assurance in bacteriology and immunology. New Delhi: World Health Organization, 2012. http://apps.searo.who.int/PDS_DOCS/B4871.pdf?ua=1.
3. Jones RN, Glick T, Sader HS, et al. Educational antimicrobial susceptibility testing as a critical component of microbiology laboratory proficiency programs: American Proficiency Institute results for 2007–2011. Diagn Microbiol Infect Dis. 2013;75(4):357-60. PMID: 23481025. https://doi.org/10.1016/j.diagmicrobio.2013.01.027.
4. Snell JJ, De Mello JV, Gardner PS. The United Kingdom national microbiological quality assessment scheme. J Clin Pathol. 1982;35(1):82-93. PMID: 7061722. PMCID: PMC497453.
5. Whitby JL, Black WA, Richardson H, Wood DE. System for laboratory proficiency testing in bacteriology: organisation and impact on microbiology laboratories in health care facilities funded by the Ontario Government. J Clin Pathol. 1982;35(1):94-100. PMID: 7061723. PMCID: PMC497454.
6. Kumasaka K, Kawano K, Yamaguchi K, et al. A study of quality assessment in clinical microbiology performance of independent laboratories in Tokyo: 18-year participation in the Tokyo Metropolitan Government External Quality Assessment Program. J Infect Chemother. 2001(2);7:102-9. PMID: 11455500. https://doi.org/10.1007/s1015610070102.
7. Siegrist HH, Pünter-Streit V, von Graevenitz A. The Swiss External Quality Assessment Scheme in Bacteriology and Mycology 1992-1996. Accreditation Qual Assur. 1998;3(5):203-07.
8. Frean J, Perovic O, Fensham V, et al. External quality assessment of national public health laboratories in Africa, 2002–2009. Bull World Health Organ. 2012(3);90:191-9A. PMCID: PMC3314205. https://doi.org/ 10.2471/BLT.11.091876.
9. Snell JJS. Problems in susceptibility testings—findings of UK NEQAS for microbiology. J Antimicrob Chemother. 1994;33:1-4. https://academic.oup.com/jac/article/33/1/1/717194.
10. Romualdez A. Department Order No. 393-E s. 2000: Designation of National Reference Laboratories and transfer of corresponding equipment, instruments, supplies, specimens, records from the Bureau of Research

and Laboratories to the designated National Reference Laboratories. November 2000. http://lcp.gov.ph/images/Dept_Order_393E_s2000.pdf.
11. Duque F. Administrative Order No. 2007-0027: Revised rules and regulations governing the licensure and regulation of clinical laboratories in the Philippines. August 2007.
12. Duque F. Department Memorandum No. 2009-0086: Implementation of External Quality Assessment Program as a regulatory requirement for licensing of clinical laboratories. February 2009.
13. Lutero N. Department Memorandum No. 2009-0086-B: Amendment to Department Memorandum No. 2009-0086-A entitled, "Implementation of External Quality Assessment Program as regulatory requirement for licensing of clinical laboratories." September 2014. http://lcp.gov.ph/images/Dept_Memo_2009_0086B.pdf.
14. O'Brien KL, Bronsdon MA, Dagan R, et al. Evaluation of a medium (STGG) for transport and optimal recovery of streptococcus pneumoniae from nasopharyngeal secretions collected during field studies. J Clin Microbiol. 2001;39(3):1021-4. PMID: 11230421. PMCID: PMC87867. https://doi.org/10.1128/JCM.39.3.1021-1024.2001.
15. Pfaller MA, Richter SS, Funke G, et al., eds. Manual of Clinical Microbiology, 11th Edition. American Society of Microbiology, 2015.
16. Atlas RM, Snyder JW. Handbook of media for clinical and public health microbiology. Boca Raton: CRC Press, Taylor and Francis Group, 2014.
17. Patel JB, Weinstein MP, Eliopoulos GM, et al. Performance standards for antimicrobial susceptibility testing. 27th ed. Clinical and Laboratory Standards Institute, 2017.
18. Dangerous goods regulations (iata--resolution 618 attachment "a"): effective 1 January-31 December 2015. Montreal: Intl Air Transport Assn, 2014.
19. Chosewood LC, Wilson DE, eds. Biosafety in microbiological and biomedical laboratories. 5th ed. Washington: U.S. Department of Health and Human Services Public Health Service Centers for Disease Control and Prevention National Institutes of Health, 2010.
20. Droettboom M, Caswell TA, Hunter J, et al. Matplotlib/Matplotlib: V2.0.0. January 2017.
21. Jones E, Oliphant T, Peterson P, et al. SciPy: Open Source Scientific Tools for Python. 2001.
22. Rodriguez-Fdez I, Canosa A, Mucientes M, Bugarin A. STAC: A web platform for the comparison of algorithms using statistical tests. In: IEEE; 2015:1-8.
23. Castillo D, Harcourt B, Hatcher C, et al. Laboratory Methods for the Diagnosis of Meningitis Caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenza, 2nd ed. Geneva, Switzerland: World Health Organisation Press, 2011.
24. Barbé B, Yansouni CP, Affolabi D, Jacobs J. Implementation of quality management for clinical bacteriology in low-resource settings. Clin Microbiol Infect. 2017;23:426-33. PMID: 28506781. https://doi.org/10.1016/j.cmi.2017.05.007.
25. Ehrmeyer SS, Laessig RH. Has compliance with CLIA requirements really improved quality in US clinical laboratories? Clin Chim Acta. 2004;346(1):37-43. PMID: 15234634. https://doi.org/10.1016/j.cccn.2003.12.033.
26. Crucitti T. National External Quality Assessment Schemes for microbiology, parasitology, and virology in Europe. Accreditation Qual Assur. 2001;6(8):379-81.
27. Libeer JC. Role of external quality assurance schemes in assessing and improving quality in medical laboratories. Clin Chim Acta. 2001;309(2):173-7. PMID: 11438297.
28. Noble MA. Advances in microbiology EQA. Accreditation Qual Assur. 2002;7(8-9):341-4.
29. Krcmery V, Barnes AJ. Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect. 2002;50(4):243-60. PMID: 12014897. https://doi.org/10.1053/jhin.2001.1151.
30. van Belkum A, Dunne WM. Next-Generation Antimicrobial Susceptibility Testing. J Clin Microbiol. 2013;51(7):2018-24. PMID: 23486706. PMCID: PMC3697721. https://doi.org/10.1128/JCM.00313-13.
31. Kiska DL, Kerr A, Jones MC, et al. Accuracy of four commercial systems for identification of Burkholderia cepacia and other gram-negative nonfermenting bacilli recovered from patients with cystic fibrosis. J Clin Microbiol. 1996;34(4):886-91. PMID: 8815102. PMCID: PMC228911.

OPEN ACCESS – REVIEW ARTICLE

An Insight into the Histopathology of Oral Neoplasms with Basaloid Morphology and a Working Classification

Manas Bajpai and Nilesh Pardhe

Department of Oral and Maxillofacial Pathology, NIMS Dental College, Jaipur, India

ABSTRACT

Basal cell tumors (BCT) are tumors usually derived from pluripotential stem cell compartments of the basal layer of epidermis and/or oral epithelium. BCTs are infrequent entities in the oral cavity and are not discussed separately in general and oral pathology. A literature review did not reveal any classification of tumors with basaloid morphology. This paper is an attempt to categorize the oral neoplasms with basaloid morphology and discuss their differential diagnoses in detail. A review of the literature was carried out to rule out the frequency of different oral BCTs reported in the literature. Additionally, a simple working classification of oral BCTS has been proposed. We hope that this classification will be helpful for oral and general pathologists and students.

Key words: basal cell tumors, basaloid morphology, oral cavity

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 21 June 2017.
Accepted: 10 August 2017.
Published online first: 8 September 2017.
https://doi.org/10.21141/PJP.2017.019

Corresponding author: Manas Bajpai, MDS
E-mail: dr.manasbajpai@gmail.com

INTRODUCTION

Basal cell tumors in the oral cavity are rare. Due to their overlapping histopathological features and admixture of basal cell and squamous cells on histopathological examination, these tumors often produce a diagnostic difficulty for pathologists and oral pathologists.[1,2,3] At times, an immunohistochemical examination is required to arrive at a final diagnosis.[4] Some authors also believe that "basaloid" patterns occurring anywhere in the body represent attempts at glandular differentiation.[5,6] An exhaustive literature review did not reveal any working classification of oral BCTs. A simple working classification of Oral BCTs is proposed here (Table 1). In this proposed working classification, oral BCTs can be classified into tumors of the oral epithelium, minor salivary gland tumors and odontogenic tumors.

Table 1. A Simple working classification proposed for oral BCTs

S. NO	Origin	Tumors
A	Tumors of Oral epithelium	1. Basal cell carcinoma. 2. Basaloid squamous cell carcinoma
B	Tumors of odontogenic epithelium	1. Basal cell ameloblastoma
C	Minor salivary gland tumors with basaloid morphology	1. Basal cell adenoma. 2. Basal cell adenocarcinoma. 3. Adenoid cystic carcinoma (solid type)

A. Tumors derived from oral epithelium

1. Intra-oral basal cell carcinoma (IOBCC)

Basal cell carcinoma is the most common adnexal tumor; however its occurrence in the oral cavity is rare and controversial.[7-10] Clinically, they present as non-healing ulcers. Histopathologically, they exhibit numerous tumor islands composed of basaloid cells in the lamina propria (Figure 1). Tumor islands exhibit a prominent palisading of peripheral basal cells (Figure 2). The diagnosis of IOBCC solely on histopathological grounds, however, is not easy, due to their histological resemblance with peripheral ameloblastoma (PA).[11,12] The differentiation between IOBCC and PA is of utmost importance since the former is malignant.

Although IOBCC and PA may be distinguished by the presence of reverse polarity in PA,[13] immunohistochemistry is considered as the

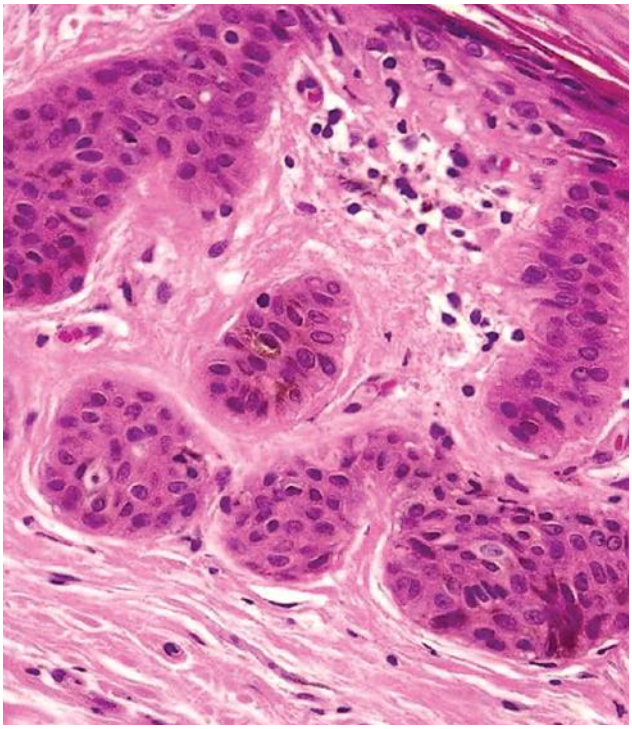


Figure 1. Tumor islands of basal cells in lamina propria (Hematoxylin and eosin stain x20).

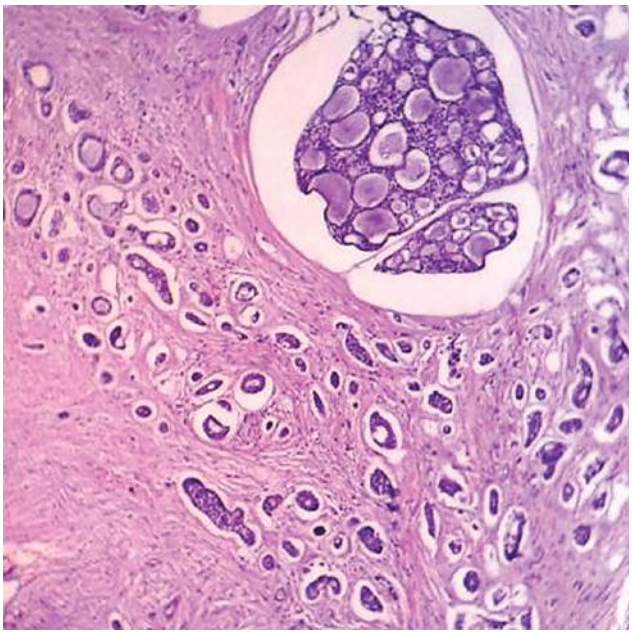


Figure 2. Tumor islands exhibit peripheral palisading (Hematoxylin and eosin stain x40).

most reliable means of differentiation. Only 21 cases of IOBCC have been reported in the literature to date.[12] Some authors believe that earlier reported cases of IOBCC were actually PA. In 2001, Del Rosario, et al.[14] reported the first well documented case of IOBCC using Ber–EP4, a tumor marker that shows a positive expression for basal cells, supporting its origin from the basal layer of the oral epithelium. Calretinin, a 29 KDa protein, has been found to show positive expression in neoplastic proliferation of ameloblastic epithelium, specifically staining the stellate reticulum-like cells of ameloblastoma.[15-17] Calretinin is considered as an additional important marker to differentiate PA from IOBCC.

2. Oral Basaloid Squamous cell carcinoma (OBSCC)

Basaloid squamous cell carcinoma (BSCC), as defined by the World Health Organization, is an aggressive, high-grade variant of squamous cell carcinoma (SCC), composed of both basaloid and squamous components.[18,19] BSCCs are common in the oropharynx but rare in the oral cavity.[20,21] Clinically, they present as indurated masses with central ulceration.[21,22] Histopathologically, they may resemble the solid variant of adenoid cystic carcinoma (ACC), adenosquamous carcinoma, and small cell neuroendocrine carcinoma (SCNC).[21-25] BSCC can be differentiated from ACC on the basis of myoepithelial cells and basement membrane-like material (both of which are found in ACC but absent in BSCC). Moreover, the atypia in ACC is less pronounced in comparison to BSCC (Figure 3).[23-25]

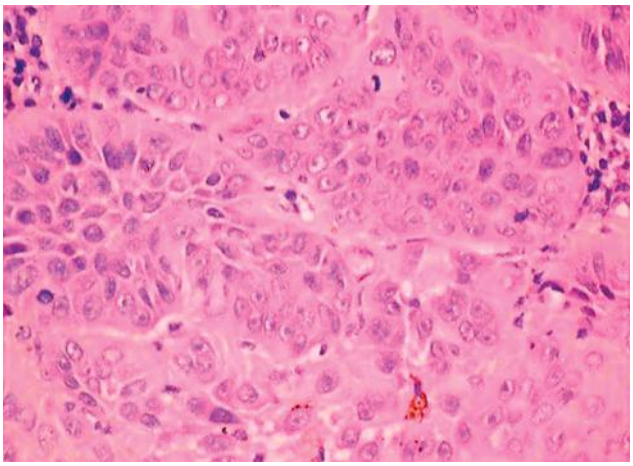


Figure 3. Infiltrating islands of basaloid cells with hyperchromatic nuclei (Hematoxylin and eosin stain x40).

Immunohistochemically, the basement membrane-like material in ACC shows positive expression for laminin and type IV collagen. P63 shows a diffuse positive expression for the tumor cells of BSCC, but is weakly expressed in ACC.[24] Adenosquamous carcinoma shows true ductal acinar differentiation and mucicarmine positivity.[23,25-27] BSCC does not show these features and can thus be differentiated from adenosquamous carcinoma.[24,26-27] On the other hand, SCNC shows nuclear molding, hyalinization and crushing artifacts on H&E, and immunohistochemically, shows positive expressions for chromogranin and synaptophysin. BSCC is devoid of all of these features.[23-24,26] Wain's criteria[27] is an essential parameter to diagnose BSCC. This criteria include both histologic (peripheral palisading associated with SCC, high nuclear-cytoplasmic ratio, high mitotic index, solid growth pattern) and immunohistochemical features (positive expression for anti-34BE1 and cytokeratin 5/6; negative expression for synaptophysin and chromogranin, and Ber-EP4). Occurrence of BSCC in the oral cavity is infrequent and very few case reports were found in the literature.[22-23,26]

A proper diagnosis of Oral BSCC is important in order to plan an appropriate treatment modality, considering its association with poor prognosis.

B. Tumors derived from odontogenic epithelium

1. Basal cell ameloblastoma (BCA)

Ameloblastomas are benign tumors whose importance lies in its potential to grow into enormous size with resulting bone deformity.[28] Basal cell ameloblastoma is a rare variant of solid

multicystic ameloblastoma (SMA) with only 11 cases reported in the literature so far.[29] Clinically they are similar to other histopathological variants of SMA.[29-31] Histopathologically, they exhibit nests of basaloid cells that show intense basophilic staining.[29] The stellate reticulum-like cells are absent and central cells may be polyhedral.[28,29] The nuclear orientation of peripheral cells is different from the other histopathological patterns of ameloblastomas: they are usually cuboidal or columnar and do not show reverse polarity (Figure 4). BCAs show a close resemblance to BCC on histopathology; however a demarcation can be made on immunohistochemical grounds by using Ber-EP4.[29,30] The prognosis and biological behavior of BCA is not clear, due to its rarity and very few reported cases.

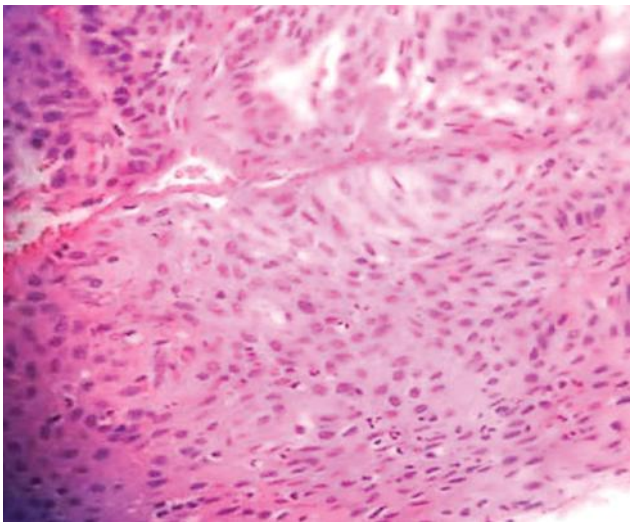


Figure 4. Sheets of basaloid cells with few cells showing highly intense basoliphic staining and peripheral cells with reversal of polarity, the sheets are separated by sparse connective tissue septa. The tissue is lacking stellate reticulum like cells which is the hallmark of ameloblastoma (Hematoxylin and eosin stain x40).

C. Tumors derived from salivary gland epithelium

1. Basal cell adenoma

Basal cell adenomas (BCA) are rare benign salivary gland tumors, most often originating from the parotid gland.[32] BCAs originating from the minor salivary glands are comparatively rare and only 13 cases have been reported in the literature. Histopathologically, they are comprised of isomorphic cells similar to basaloid cells with palisading arrangement and distinctive basement membrane-like material (Figure 5). BCAs share a close resemblance with canalicular adenoma and Basal cell adenocarcinomas (BCAC). The trabecular–tubular variant of BCA may be misdiagnosed as canalicular adenoma on low power view, and a proper inspection on high power exhibits the presence of both basal and luminal cells with more collagenized stroma compared to canalicular adenoma.[33,34] BCACs, on the other hand, are not encapsulated and tend to invade the underlying connective tissue stroma.[34,35]

2. Basal cell adenocarcinoma

Basal cell adenocarcinomas (BCAC) represent the malignant counterpart of basal cell adenoma, believed to arise from pluripotent ductal reserve cells.[36-38] Histopathologically, they are characterized by sheets of basaloid cells with hyperchromatic nuclei (Figure 6). Some sheets show peripheral palisading. Some tumors show nests invading the connective tissue stroma. It is necessary to differentiate BCAC from other basaloid cell tumors because of the differences in prognosis and potential differences in treatment.

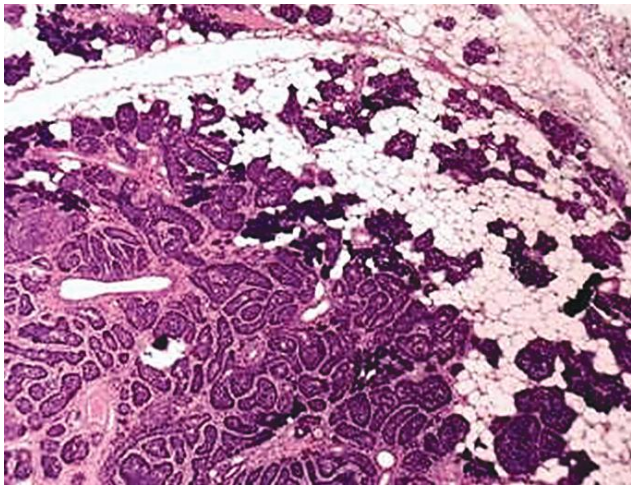


Figure 5. Isomorphic cells similar to basaloid cells with palisading arrangement and distinctive basement membrane like material (Hematoxylin and eosin stain x40).

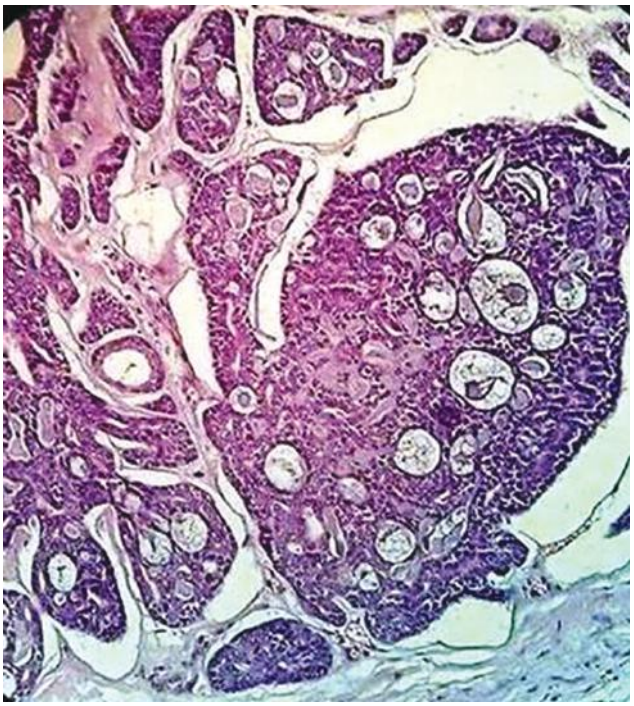


Figure 6. Isomorphic cells similar to basaloid cells with palisading arrangement and distinctive basement membrane like material (Hematoxylin and eosin stain x40).

3. Adenoid cystic carcinoma

Adenoid cystic carcinoma (ACC) was originally described by Lorain and Laboulbene in 1853. In 1859, Billroth suggested the name cylindroma.[39] ACC is a rare tumor, accounting for less than 1% of all head and neck neoplasms and about 4 – 10 % of all salivary gland neoplasms. Histopathologically, ACC shows three different patterns, tubular, cribriform and solid.[40] The solid pattern of ACC is believed to be the most aggressive form with poor prognosis.[39] Solid ACC is characterized histopathologically by tumor nests or islands completely filled with basaloid cells without cystic spaces.

CONCLUSION

It can be concluded that oral BCTs are unusual and may represent diagnostic difficulties on microscopic examination because of their overlapping features. This paper is an attempt to discuss the histopathology and differential diagnosis of various oral neoplasms with basaloid morphology. A simple working classification of oral BCTs has been proposed with the aim of helping pathologists distinguish between various oral entities with basaloid features.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Tellechea O, Reis JP, Domingues JC, Baptista AP. Monoclonal antibody Ber-EP4 distinguishes basal-cell carcinoma from squamous- cell carcinoma of the skin. Am J Dermatopathol. 1993;15(15):452–5. PMID: 8238781.
2. Nemade SV, Rokade VV. Basaloid carcinoma: an unusual presentation. Indian J Clin Pract. 2013; 23(11):728-30. http://imsear.hellis.org/handle/123456789/182519.
3. Nagao T, Sugano I, Ishida Y, Hasegawa M, Matsuzaki O, Konno A, et al. Basal cell adenocarcinoma of the salivary glands: comparison with basal cell adenoma through assessment of cell proliferation, apoptosis, and expression of p53 and bcl-2.Cancer. 1998; 82:439-47. PMID: 9452259.
4. Carr RA, Taijbee SM, Sanders DSA. Basaloid skin tumours: basal cell carcinoma. Curr Diagn Pathol. 2007; 13(4):252–72. https://doi.org/10.1016/j.cdip.2007.05.005.
5. Basarab T, Orchard G, Russell-Jones R. The use of immunostaining for bcl-2 and CD34 and the lectin peanut agglutinin in differentiating between basal cell carcinomas and trichoepitheliomas. Am J Dermatopathol 1998; 20(5):448–52. PMID: 9790103.
6. Thariat J, Badoual C, Faure C, Butori C, Marcy PY, Righini CA. Basaloid squamous cell carcinoma of the head and neck: role of HPV and implication in treatment and prognosis. J Clin Pathol. 2010; 63(10):857–66. PMID: 20876315. https://doi.org/10.1136/jcp.2010.078154.
7. Bajpai M, Pardhe N, Arora M. Intra–oral basal cell carcinomaan immunohistochemical interpretation using Ber-Ep4. J Ayub Med Coll Abbottabad. 2016; 28(2):438. PMID: 28718582.
8. Mori M, Morimoto Y, Yoshimura Y, Kawamura H. Enzymatic histochemical demonstration of basal-cell carcinoma in the oral cavity. Oral Surg Oral Med Oral Pathol. 1968; 25(5):746–55. PMID: 5239097.
9. Bath-Hextall F, Leonardi-Bee J, Smith C, Meal A, Hubbard R. Trends in incidence of skin basal cell carcinoma. Additional evidence from a UK primary care database study. Int J Cancer. 2007; 121(9):2105–8. PMID: 17640064. https://doi.org/10.1002/ijc.22952.
10. Liroff KP, Zeff S. Basal cell carcinoma of the palatal mucosa. J Oral Surg. 1972; 30(10):730-3. PMID: 4506539.
11. Bajpai M, Pardhe N. Peripheral ameloblastoma with mixed histological patterns. Cukurova Med J. 2015; 40(Suppl 1):151–5. https//doi.org/10.17826/cutf.32647.
12. Woods TR, Cohen DM, Islam MN, Kratochvil FJ, Stewart JC, Reeder SL, et al.. Intraoral basal cell carcinoma, a rare neoplasm: report of three new cases with literature review. Head Neck Pathol. 2014; 8(3):339-48. PMID: 24202723. PMCID: PMC4126916. https://doi.org/10.1007/s12105-013-0505-5.
13. Philipsen HP, Reichart PA, Nikai H, Takata T, Kudo Y. Peripheral ameloblastoma: biological profile based on 160 cases from the literature. Oral Oncol. 2001; 37(1):17-27. PMID: 11120479.
14. Del Rosario RN, Barr RJ, Jensen JL, Cantos KA. Basal cell carcinoma of the buccal mucosa. Am J Dermatopathol. 2001; 23(3):203–5. PMID: 11391100.
15. DeVilliers P, Liu H, Suggs C, Simmons D, Daly B, Zhang S, et al. Calretinin expression in the differential diagnosis of human ameloblastoma and keratocystic odontogenic tumor. Am J Surg Pathol. 2008; 32(2):256-60. PMID: 18223328. https://doi.org/10.1097/PAS.0b013e3181452176.
16. Alaeddini M, Etemad-Moghadam S, Baghaii F. Comparative expression of calretinin in selected odontogenic tumours: a possible relationship to histogenesis. Histopathology. 2008; 52(3):299-304. PMID: 18269580. https://doi.org/10.1111/j.1365-2559.2007.02948.x.
17. Anandani C, Metgud R, Singh K. Calretinin as a diagnostic adjunct for ameloblastoma. Pathol Res Int. 2014; 2014:308240. PMID: 24839578. PMCID: PMC4009281. https://doi.org/ 10.1155/2014/308240.
18. Ide F, Shimoyama T, Horie N. Basaloid squamous cell carcinoma versus basal cell ameloblastoma. Oral Oncol. 1998; 34(2):154-5. PMID: 9682780.
19. Greene FL, Page DL, Fleming ID, eds. AJCC/UICC cancer staging handbook: TNM classification of malignant tumors. 7th ed. New York: Springer-Verlag, 2009.
20. Ereño C, Gaafar A, Garmendia M, Etxezarraga C, Bilbao FJ, López JI. Basaloid squamous cell carcinoma of the head and neck: a clinicopathological and follow-up study of 40 cases and review of the literature. Head Neck Pathol. 2008; 2(2):83–91. PMID: 20614328. PMCID: PMC2807543. https://doi/org/10.1007/s12105-008-0045-6.
21. Patil AV, Tupsakhare SD, Shah K, Gabhane M, Ali FM. Basaloid squamous cell carcinoma of gingiva: a new case and review of literature. Am J Cancer Case Rep. 2014; 2:99-107.
22. Sundharam BS, Krishnan PA. Basaloid squamous cell carcinoma report of a case and review of literature. Indian J Dent Res. 2003;14(3):184-6. PMID: 15164662.
23. Ide F, Shimoyama T, Horie N. Kusama K. Basaloid squamous cell carcinoma of the oral mucosa: a new case and review of 45 cases in the literature. Oral Oncol. 2002; 38(1):120-4. PMID: 11755833.
24. Emanuel P, Wang B, Wu M, Burstein DE. Immunohistochemistry in the distinction of adenoid cystic carcinoma from basaloid squamous cell carcinoma. Mod Pathol. 2005; 18(5):645-50. PMID: 15529180. https://doi.org/10.1038/modpathol.3800329.
25. Begum S, Westra WH. Basaloid squamous cell carcinoma of the head and neck is a mixed variant that can be further resolved by HPV status. Am J Surg Pathol. 2008; 32(7):1044–50. PMID: 18496144. https://doi.org/10.1097/PAS.0b013e31816380ec.
26. Vasudev P, Boutross-Tadross O, Radhi J. Basaloid squamous cell carcinoma: two case reports. Cases J. 2009; 2:9351.

PMID: 20062602. PMCID: PMC2804002. https://doi.org/10.1186/1757-1626-2-9351.
27. Wain SL, Kier R, Vollmer RT, Bossen EH. Basaloid-squamous carcinoma of the tongue, hypopharynx, and larynx: report of 10 cases. Hum Pathol. 1986; 17(11):1158–66. PMID: 3770734.
28. Bajpai M, Agarwal D, Bhalla A, Kumar M, Garg R, Kumar M. Multilocular unicystic ameloblastoma of mandible. Case Rep Dent 2013; 2013:835892. PMID: 24106618. PMCID: PMC3782764. https://doi.org/ 10.1155/2013/835892.
29. Kramer IR, Pindborg JJ, Shear M. histological typing of odontogenic tumors, 2nd ed. Berlin: Springer-Verlag, 1992.
30. Saify F, Sharma N. Basal cell ameloblastoma: a rare case report and review of literature. Oral Maxillofac Pathol J 2010; 1:1-7. http://www.ompj.org/files/b9724b4e67cc8a4760a2004b52b6550a-fatima.pdf.
31. Shakya H, Khare V, Pardhe N, Mathur E, Chouhan M. Basal cell ameloblastoma of mandible: a rare case report with review. Case Rep Dent. 2013; 2013:187820. PMID: 23653864. PMCID: PMC3638548. https://doi.org/10.1155/2013/187820.
32. Chiu NC, Wu HM, Chou YH, Li WY, Chiou YY, Guo WY, et al. Basal cell adenoma versus pleomorphic adenoma of the parotid gland. AJR Am J Roentgenol. 2007; 189(5): W254-1. PMID: 17954621. https://doi.org/10.2214/AJR.07.2292.
33. Batsakis JG, Luna MA, el-Naggar AK. Basaloid monomorphic adenomas. Ann Otol Rhinol Laryngol. 1991; 100(8):687–90. PMID: 1872522. https://doi.org/10.1177/000348949110000818.
34. Bajpai M. Canalicular adenoma arising in buccal mucosa. J Coll Physician Surg Pak. 2016; 26(11):46. PMID: 27981939.
35. Fonseca I, Soares J. Basal cell adenocarcinoma of minor salivary and seromucous glands of the head and neck region. Semin Diagn Pathol. 1996; 13(2):128-37. PMID: 8734419.
36. Hirsch DL, Miles C, Dierks E. Basal cell adenocarcinoma of the parotid gland: report of a case and review of the literature. J Oral Maxillofac Surg. 2007; 65(11):2385-8. PMID: 17954345. https://doi.org/10.1016/j.joms.2006.07.021.
37. Parashar P, Baron E, Papadimitriou JC, Ord RA, Nikitakis NG: Basal cell adenocarcinoma of the oral minor salivary glands: review of the literature and presentation of two cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007, 103(1):77-84. PMID: 17178498. https://doi.org/10.1016/j.tripleo.2005.12.021.
38. Jung MJ, Roh JL, Choi SH, Nam SY, Kim SY, Lee SW, et al. Basal cell adenocarcinoma of the salivary gland: a morphological and immunohistochemical comparison with basal cell adenoma with and without capsular invasion. Diagn Pathol. 2013; 8:171. PMID: 24143938. PMCID: PMC4016524. https://doi.org/10.1186/1746-1596-8-171.
39. Martínez-Rodríguez N, Leco-Berrocal I, Rubio-Alonso L, Arias-Irimia O, Martínez-González JM. Epidemiology and treatment of adenoid cystic carcinoma of the minor salivary glands: a meta-analytic study. Med Oral Patol Oral Cir Bucal. 2011; 16(7):e884-9. PMID: 21743416.
40. Eneroth CM, Hjertman L, Moberger G. Adenoid cystic carcinoma of the palate. Acta Otolaryngol.1968; 66(3):248–60. PMID: 4303189.

REGISTRATION IS FREE ● LIMITED SLOTS ONLY

Researchers in health sciences and health social sciences not more than 35 years old who have completed a research project and have drafted a manuscript for submission to a scholarly journal are invited to join!

For more information, visit www.pchrd.dost.gov.ph

** This advertisement is a complimentary service of the PJP for member societies/organizations.*

OPEN ACCESS – CASE REPORT

The Morphologic Profile of Inflammatory Bowel Disease and the Diagnostic Problem of Crohn's Disease versus TB Colitis – A Case Series

Maria Lourdes Tilbe, Francia Victoria De Los Reyes, Ricka Cu, Kathleen Perez

University of the East Ramon Magsaysay Memorial Medical Center, Quezon City, Philippines

ABSTRACT

The aim of this study is to describe the morphologic profile of the biopsy and resection specimen that were diagnosed with Crohn's disease and ulcerative colitis in the University of the East Ramon Magsaysay Memorial Medical Center (UERMMMC) from 2008-2016. Features that classify the specimen as Inflammatory Bowel Disease – Indeterminate Type are also presented. Considerations for the definitive IBD classification after an initial indeterminate diagnosis by morphology are also briefly discussed. Biopsy and resection specimen that were diagnosed with Crohn's disease, cannot exclude TB Colitis, are also presented; and the subsequent steps for a definitive classification are also discussed. All the patients included underwent an endoscopic biopsy, and are categorized by histopathologic diagnoses, age, sex, and GIT segment involved in the endoscopic procedure. Patients that underwent subsequent resection due to the disease condition are also identified.

Comparison of the histologic findings observed in the patients, with the microscopic basis for the diagnosis recommended by the European consensus on the histopathology of inflammatory bowel disease (2013), and with the histologic features described by Patil et al., (2015) for the inflammatory disorders of the large intestine, is done. The histologic features described by Lamps (2015) for the gastrointestinal TB is used in the evaluation of the findings in the patients diagnosed with Crohn's disease, cannot exclude TB Colitis.

There are 5 Crohn's disease patients, accounting for 0.8% of all patients with lower GIT inflammatory conditions, and 10 ulcerative colitis patients, accounting for 1.6% of all patients with lower GIT inflammatory conditions. Seven patients, which comprise 1.1% of all patients with lower GIT inflammatory conditions, have the diagnosis of indeterminate colitis. The histologic features of 6 out of 7 patients that had the initial diagnosis of indeterminate colitis presented with morphologic features that favored an ulcerative colitis, but with Crohn's disease features. In comparison, one patient who had an initial diagnosis of indeterminate colitis presented with morphologic features that favored Crohn's disease but with ulcerative colitis features. In these patients, correlation with chronology of symptoms and associated ancillary procedures that can classify the patients as CD or UC are recommended to the gastroenterologist attending such patients so that a more definitive classification can be done.

Four patients, accounting for 0.6% of all lower GIT inflammatory conditions, were initially diagnosed as Crohn's disease, cannot exclude TB Colitis. This is in contrast with 34 patients who were diagnosed with Chronic Granulomatous Inflammation, Tuberculosis which accounted for 5.7% of all patients that were diagnosed with inflammatory conditions of the lower GIT. The remaining 536 patients were composed of acute self-limited/ infectious colitis, ischemic colitis, eosinophilic colitis, inflammatory polyp, and nonspecific inflammation.

With the trend of increasing incidence of Inflammatory Bowel Disease in Asia, comparison of the more commonly seen causes of chronic inflammation of the gastrointestinal tract with a condition that appears to have a growing incidence in the region is necessary for optimal diagnostic protocol, management, and quality of care.

Key words: inflammatory bowel disease, Crohn's disease, ulcerative colitis, indeterminate colitis, intestinal tuberculosis, colitis, morphology, histopathology

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 1 April 2017.
Accepted: 3 June 2017.
Published online first: 7 August 2017.
https://doi.org/10.21141/PJP.2017.013

Corresponding author: Francia Victoria A. De Los Reyes, MD
E-mail: kaidelosreyes@gmail.com

INTRODUCTION

Inflammatory bowel disease (IBD), comprising of the two disorders ulcerative colitis (UC) and Crohn's disease (CD), is a disease condition that results from a chronic, inappropriate immune activation in the mucosa with subsequent involvement of the submucosa for ulcerative colitis, and full-thickness wall involvement for Crohn's disease. Although known to be more frequent in North America, northern Europe, and Australia, there is a trend of increasing incidence documented in Asia, Africa, and South America.

Hypothesis regarding the role of the luminal gut microbiota and the changes in gut microbiome composition due to improved

food storage condition, and decreased food contamination of previously documented commonly occurring food-borne infectious organisms have been described. The role of changes in the gut microbial flora triggers a cascade of persistent inflammation in susceptible hosts that manifest as chronic gastrointestinal symptoms. Although, much is yet to be discovered regarding the mechanisms of this.[1]

Intestinal tuberculosis (TB), on the other hand, is a disease condition that has a high disease burden in developing countries. To apply this, in the Philippines, TB is the sixth leading cause of morbidity and mortality, and is the ninth out of the 22 highest TB-burden countries in the world. The country likewise has one of the highest burdens of multidrug-resistant TB.[2] Intestinal TB belongs under the broader category of abdominal TB, which has been documented to constitute about 12% of extra-pulmonary TB and 1%–3% of the total TB cases.[3] However, no multicenter study in the country documenting the trend of incidence has been documented to specify the incidence or prevalence of intestinal TB alone versus all other abdominal TB.

Both of the two disease conditions, IBD and intestinal TB have features constituting chronic inflammation, segmental or diffuse involvement, prolonged treatment requirement, and significant interplay of host immune-response with gut microbiome-related injury. Comparatively, it has been documented that there is an overlap in the genes responsible for the immune-mediated mechanism of IBD and the immune response to mycobacteria infection, including *Mycobacterium tuberculosis* and *Mycobacterium leprae*.[1,4] As such, comparison of the morphologic features of IBD and intestinal TB may be necessary in countries where there is an increasing incidence of one and a consistently high incidence of the other.

To address such concern in our local setting, this paper describes the morphologic features of the biopsy and/or resection specimen of the patients diagnosed with CD, UC, and IBD-Indeterminate (IBD-IC), and CD vs TB colitis in the University of the East Ramon Magsaysay Memorial Medical Center from 2008-2016. The aim of comparing the morphologic features of IBD and indicating instances when TB cannot be completely excluded is so that there is an initial groundwork for establishing an algorithm that can be used in diagnosing IBD and excluding TB, or concluding a concurrent TB infection given a particular morphologic pattern.

CASE

To determine the prevalence of IBD in our institution, we reviewed all the surgical pathology reports in the UERMMMC Pathology Laboratory database from 2008-2016 and determined the occurrence of the various morphologic diagnosis of the inflammatory conditions of the lower gastrointestinal tract. The census was generated using the morphologic diagnosis that was reported in our institution for the past 8 years, and were compared with the morphologic findings used by Odze et al., in

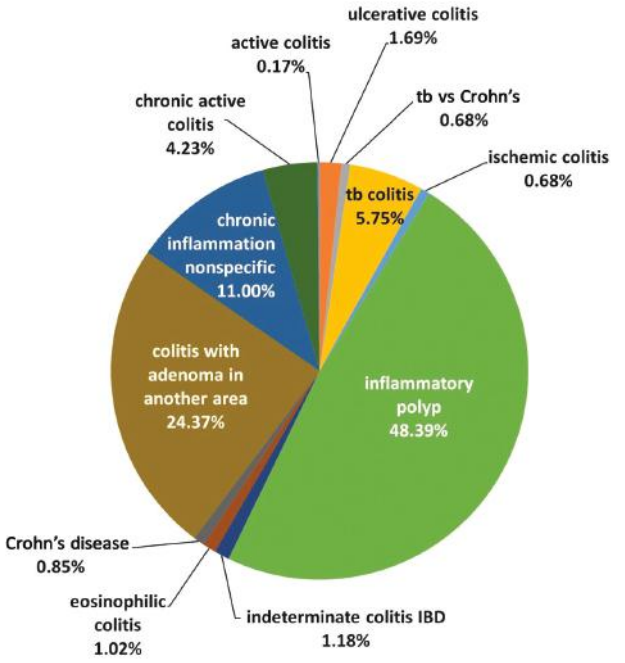


Figure 1. Inflammatory diseases of the ileum, colon, and anorectal region in the UERMMMC Pathology Laboratory from 2008 to 2016 (n=596).

2015 discussing the disorders of the GIT to ensure the timeliness of reporting such morphologic features. The census included inflammatory polyp, chronic nonspecific inflammation, active colitis, chronic active colitis, ischemic colitis, eosinophilic colitis, diagnosis of colitis with an adenoma seen in another segment of the GIT but with no malignancy, TB colitis, CD, UC, IBD-indeterminate, and CD but TB colitis cannot be completely excluded. All reports with the surgical pathology diagnosis of acute appendicitis, ruptured viscus that was not secondary to a known chronic inflammatory condition, post-operative complications, diverticular disease, and malignancy-associated lesions without a previously documented association with IBD, were excluded. The patients with the diagnosis of CD, UC, IBD-indeterminate, CD but cannot exclude TB were described in terms of the male-to-female ratio, age, and GIT segment involved in the endoscopic or surgical resection procedures (Table 1).

Comparison of the morphologic findings of the included reports, with the microscopic basis for the diagnosis recommended by the European consensus on the histopathology of inflammatory bowel disease of 2013,[5] and with the histologic features described by Patil et al., in 2015 for the inflammatory disorders of the large intestine, and the histologic features described by Lamps in 2015 for the gastrointestinal TB, were done.

Using these parameters, a total of 596 patients that were diagnosed with inflammatory conditions of the lower gastrointestinal tract were included in the census.

Table 1. Characteristics of IBD patients and intestinal TB vs. CD patients

Diagnosis	Number of Cases	Age Mean (Range)	M/F	Site of Involvement by Frequency
Crohn's Disease	5	35.4 (17-74)	5/0	ileum > stomach, cecum, ascending colon, descending colon, rectum
Ulcerative Colitis	10	49 (25-77)	5/5	sigmoid colon > rectum > descending colon > ascending colon, transverse colon > cecum, anastomotic site (s/p right hemicolectomy)
Indeterminate Colitis	7	48.8 (17-72)	5/2	cecum > rectum > ileum,transverse colon, sigmoid colon > anal canal
Intestinal TB vs. Crohn's Disease	4	39.5 (26-66)	4/0	Ileum > cecum, ascending colon, transverse colon, descending colon, rectum

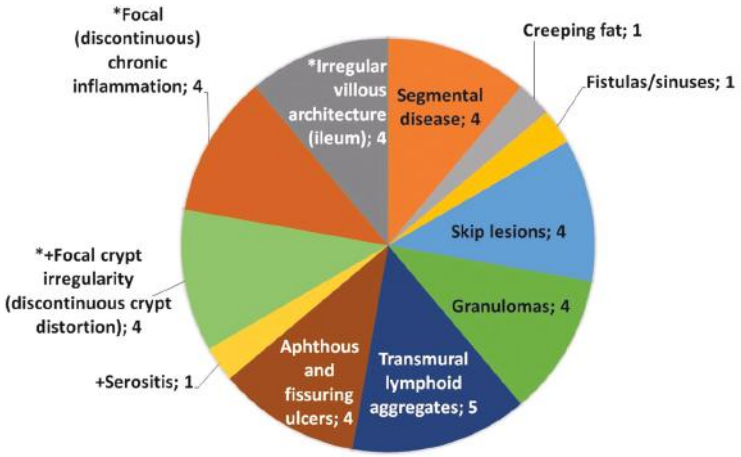


Figure 2. Frequency of specific morphologic features in Crohn's disease (n= 5).
+ minor morphologic criteria Patil et al., 2015.
** morphologic criteria European Consensus on the histopathology of Crohn's disease, 2013*

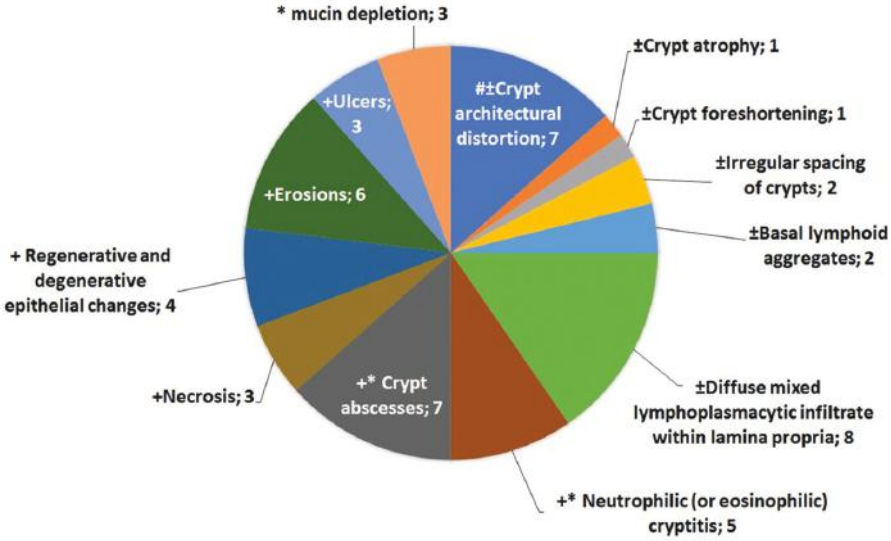


Figure 3. Frequency of specific morphologic features in ulcerative colitis (n=10).
± histologic features of chronicity of UC lesion Patil et al., 2015,
+ histologic features of activity of UC Patil et al., 2015,
** morphologic criteria for active European Consensus on the histopathology of ulcerative colitis, 2013*

Among all of these, only 5 patients were diagnosed with Crohn's disease, accounting for 0.8% of all included patients. The patients diagnosed with Crohn's disease were all male, with the age range of 17-74 years old, and the sites involves are the following in descending order of frequency: ileum in 4 patients, and stomach, cecum, ascending colon, descending colon, and rectum with involvement found in 1 patient each. The most common major pathologic findings include transmural lymphoid aggregates, segmental disease, granulomas, fistulas, and fissuring ulcers. Only focal crypt irregularity among the secondary pathologic features is consistently observed. All the major pathologic features indicated by Patil et al., and all the features enumerated under the 2013 consensus are observed in the patients (Figure 2).

In contrast to CD, ulcerative colitis was observed in 10 patients, and this comprised 1.6% of the population included. In this group, there is a 1:1 male-to-female ratio, and the age range is similarly wide like in CD, with the patients' ages ranging from 25 to 77 years old. The sites involved are the following, in descending order of frequency: sigmoid colon with 6 patients noting involvement, rectal involvement in 5 patients, descending colon involvement in 3 patients, ascending colon and transverse colon involvement in 2 patients, cecal involvement in 1 patient, and an anastomotic site in a status post right hemicolectomy patient in 1 patient. For all patients diagnosed with ulcerative colitis, the most commonly observed features include diffuse mixed lymphocytic and plasmacytic infiltrate within the lamina propria, crypt abscess, and crypt architectural distortion. All of the patients presented with a chronic active type of disease with no fibrosis observed (Figure 3).

Seven patients, comprising 1.1% of the included population, were given the diagnosis of indeterminate colitis. Among the patients diagnosed with Inflammatory Bowel Disease-indeterminate colitis, the age range is 17-72 years old, and a 2.5:1 male-to-female ratio was noted. The sites involved in descending order of frequency included cecum in 4 patients, rectum in 3 patients, ileum in 2 patients, transverse colon in 2 patients, sigmoid colon

in 2 patients, and anal canal in 1 patient. The histologic features of 6 out of 7 indeterminate colitis favored ulcerative colitis, and only one patient had a morphologic diagnosis that favored Crohn's disease more than ulcerative colitis.

Although it is important to note that indeterminate colitis is an interim diagnosis that requires further work-up for a definitive classification, it is equally important for us to determine what kind of pitfalls were present during the histopathologic evaluation of such specimen and identify the morphologic features that precludes the definitive classification of CD or UC. Morphologic Diagnosis of IBD-Indeterminate type in the UERMMMC is an interim diagnosis and made only after exclusion of all other causes of colitis and the biopsy clearly has morphologic features pertaining to an IBD morphology, and when only one or two features of chronicity and one or two features of activity for ulcerative colitis were observed.

The most commonly observed features that caused the diagnosis of indeterminate colitis were divided into ulcerative colitis with Crohn's-like features, and Crohn's disease with ulcerative colitis–like features. Crohn's disease with UC-like features were noted as persistently superficial fissuring ulcers in a discontinuous disease with anal fistula formation, but with no features indicating granuloma formation, transmural lymphoplasmacytic inflammation, or lymphoid hyperplasia. In contrast, UC with CD–like features were noted in those with superficial mucosal involvement which manifests as mucosal ulceration with absent granuloma formation but is discontinuous and has lymphoid hyperplasia with no cryptitis. In these patients, correlation with the clinical course and ancillary laboratory tests that can provide information whether the associated features of the patient's gastrointestinal lesion were more likely associated with CD or UC were done in coordination with the attending gastroenterologist so that a more definitive classification can be made. In patients with more morphologic features consistent with CD, treatment for CD was initiated by the attending gastroenterologist, and treatment for UC was initiated in patients with more morphologic features consistent with UC. In indeterminate colitis, patients with CD-like lesions but lack granuloma or transmural inflammation may have undergone biopsy prior to the transmural involvement or development of granuloma of the segment sampled. In comparison, patients with UC-like features but have patchy disease may belong to the small population of UC with an initially segmental disease because there is limited involvement of particular colon segments, for example a diffuse involvement of the left side of the colon with a patchy involvement of the right side of the colon, which will become confluent towards the progression of UC into a diffuse involvement.

Also documented were 4 patients, accounting for 0.6% of all the patients included, have the diagnostic problem of CD, cannot totally rule out TB colitis. This is interpreted in the setting of a greater prevalence of clear cut TB colitis patients with 34 patients, comprising of 5.7% of all included patients, diagnosed with intestinal TB. The work-up of all patients with the morphologic diagnosis of CD cannot exclude TB were reviewed for the history of active or latent TB, status of ancillary testing for Mycobacterium TB such as PCR of endoscopic biopsy specimen and AFP special stain, taken at the same time as the specimen with the problematic diagnosis. In these patients, the age range is 26-66 years old, all patients are males, and the sites involves are the following in descending order of frequency: ileum in 3 patients, and 1 patient each for cecum, ascending colon, transverse colon, descending colon, and rectum. All four patients with chronic granulomatous inflammation, consider TB colitis versus Crohn's disease show focal and discontinuous chronic inflammation. No discontinuous crypt distortion and no irregular villous architecture were seen. One patient also showed a lesion in the ileum with granulomas with multinucleated giant cells. In such situation, the consideration of intestinal TB was entertained due to the high incidence of the disease in the country. All the biopsy specimen also showed no Mycobacterium tuberculosis bacilli on AFB stain. Clinical evaluation also showed no evidence of an acute or latent TB infection in all four patients, and as such were managed for CD.

However, one patient that had been undergoing treatment for CD for three months presented with an exacerbation of CD and underwent right hemicolectomy. The resection specimen showed positive Mycobacterium tuberculosis bacilli in the areas exhibiting caseation necrosis, whereas the biopsy showed negative findings on MTB PCR and AFB special stain in the specimen from the cecum and the rectum which was done three month prior to the exacerbation. In this patient, there was non-caseation necrosis in the biopsy specimen of the cecum, and in the subsequent sampling of the cecum after right hemicolectomy was done, but caseation necrosis was noted in the transverse colon segment of the resection specimen. The conversion from a previously negative to a positive TB involvement may indicate a latent TB infection that was activated upon immunosuppression during the course of treatment for Crohn's disease.[6]

DISCUSSION

The diagnosis of IBD requires the correlation of endoscopic findings that provides information on the gross features of the intestinal architecture, such as luminal caliber, distensibility, and macroscopic mucosal lesions, with the morphologic features noted in the histopathologic evaluation because even grossly unremarkable looking mucosa may harbor critical features that clinch the diagnosis of CD or UC. It is equally important to emphasize that multiple slide sections from the sampled segments are required and although there is no consensus as of the publication of Langer et al., in 2014 regarding the number of sections required for an adequate diagnosis, the recommendation of step-sections of two to three tissue levels, having five or more sections is useful to exhaust the features which may otherwise not be visualized if there is a limited sampling.[7] This is particularly useful in our institution when faced with vague features such as poorly formed granulomas, or noncaseating granulomas in a resection specimen with another lesion that has a caseating granuloma.

Extraintestinal manifestations of IBD are also important but more so because having a particular IBD increases the chance of a patient developing a disease associated with one type versus another, for example Primary Sclerosing Cholangitis is more likely if the patient has ulcerative colitis than if the patient has Crohn's disease. Although if a patient presents with symptoms of rectal bleeding, mucoid discharge from the rectum, frequent stools, and associated liver disease manifesting as cholestasis, the diagnosis of ulcerative colitis is highly likely and quite warranted. However, the cornerstone of diagnosing IBD is still clinical constellation of symptoms, endoscopic findings, and morphologic features. As such, documenting the morphologic features that allows institutions to create an algorithm that can streamline the evaluation of chronic inflammatory lesions that

may have mimics is necessary so that a standardized system based on morphologic parameters established in published literature can be used. Also, documenting problematic situations unique to regions with infectious processes that mimics IBD or complicates IBD may help in addressing future concerns related to such mimics. The standardized system based on morphologic parameters should incorporate ruling out TB when the classic morphology of TB is not seen.

Although this study is limited by the lack of information on the gross findings seen on endoscopic evaluation, the inclination of this study is to establish the morphologic features based on histopathology and to discuss the features that influence favoring the diagnosis of one specific lesion versus another when a case is under a larger general category.

Future studies to determine the incidence of CD and UC, and its association with TB is recommended to determine whether there is an increasing trend of IBD and whether IBD treatment will lead to a higher occurrence of TB reactivation.

CONCLUSION

Given that there is an increasing trend of IBD in Asia, there should be a working system to classify such patients based on standardized morphologic parameters based on published literature that also reflect the current practices in areas with a higher incidence of IBD. Likewise, the algorithm for diagnosis should incorporate ruling out TB when the classic morphology of TB is not seen, and should address the development of active TB in those with latent TB infection that had undergone treatment for IBD. With the trend of increasing incidence of Inflammatory Bowel Disease in Asia, comparison of the more commonly seen causes of chronic inflammation of the gastrointestinal tract, particularly TB, with a condition that appears to have a growing incidence in the region, such as IBD, is necessary for optimal management and improved quality of care.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support of the faculty and staff of the Department of Pathology, College of Medicine, University of the East Ramon Magsaysay Memorial Medical Center, and the Section of Histopathology, Pathology Laboratory of the University of the East Ramon Magsaysay Memorial Medical Center.

ETHICAL CONSIDERATION

This case report has no studies performed to animal or human participants. This case report includes only the specimen submitted by the patient for surgical histopathology evaluation to the section of anatomic pathology with full, informed consent of specimen evaluation and non-profit academic discussion or report.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors have declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Turner JR. The Gastrointestinal Tract. In: Kumar V, Abbas AK, Aster JC ed. Robbins and Cotran Pathologic Basis of Disease, 9th ed. Philadelphia: Elsevier-Saunders; 2015: 796-800.
2. Vianzon R, Garfin AMC, Lagos A, Belen R. The tuberculosis profile of the Philippines, 2003–2011: advancing DOTS and beyond. Western Pacific Surveillance and Response Journal. 2013;4(2):11-6. https://doi.org/10.5365/wpsar.2012.3.4.022.
3. Sheer TA, Coyle WJ. Gastrointestinal tuberculosis. Curr Gastroenterol Rep. 2003;5(4):273-8. PMID: 12864956.
4. McNees A, Markesich D, Zayyani N, Graham D. Mycobacterium paratuberculosis as a cause of Crohn's disease. Expert Rev Gastroenterol Hepatol. 2015;9(12):1523-34. PMCID: PMC4894645. NIHMSID: NIHMS787513. https://doi.org/10.1586/17474124.2015.1093931.
5. Magro F, Langner C, Driessen A, et al. European consensus on the histopathology of inflammatory bowel disease. J Crohns Colitis. 2013;7(10):827-51. https://doi.org/10.1016/j.crohns.2013.06.001.
6. Ye L, Liu J, Lin Z, Cao Q. Does infliximab therapy increase incidence of tuberculosis in patients with inflammatory bowel disease in an endemic area: a nationwide study from China. Poster Presentation to the 12th Congress of European Crohn's and Colitis Organization, Barcelona 2017. https://www.ecco-ibd.eu/index.php/publications/congress-abstract-s/abstracts-2017/item/p568-does-infliximab-therapy-increase-incidence-of-tuberculosis-in-patients-with-inflammatory-bowel-disease-in-an-endemic-area-a-nationwide-study-from-china-2.html.
7. Langer C, Magro F, Driessen A, Ensari A, et al. The histopathological approach to inflammatory bowel disease: a practice guide. Virchows Arch. 2014;464(5):511-27. PMID: 24487791. https://doi.org/10.1007/s00428-014-1543-4.

Intravascular Large B-Cell Lymphoma: A Continuing Clinical Enigma

Raymundo Lo[1] and Ivy Carol Lique[2]

[1]*Department of Laboratories, Cardinal Santos Medical Center*
[2]*Division of Pathology, Philippine Children's Medical Center*

ABSTRACT

Intravascular large B-cell lymphoma (IVLBCL) is a rare form of extranodal diffuse large B-cell lymphoma characterized by the proliferation of lymphoma cells in the lumen of small blood vessels. Clinical presentation varies among reported cases and diagnosis can be challenging for both clinicians and pathologists. We report a case of a 64-year-old female with a history of prolonged fever. Diagnosis was suspected clinically due to thrombocytopenia and elevated serum lactate dehydrogenase after exhausting work-up for an infectious etiology. IVLBCL was established on post-mortem examination upon finding infiltration of CD20-positive neoplastic cells in several organs in the absence of lymph node involvement.

Key words: lymphoma, large B cell, intravascular, fever of unknown origin

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 27 July 2017.
Accepted: 22 August 2017.
Published online first: 24 August 2017.
https://doi.org/10.21141/PJP.2017.014

Corresponding author: Ivy Carol A. Lique, MD
E-mail: aibi.lique@gmail.com

INTRODUCTION

Intravascular large B-cell lymphoma (IVLBCL) is a rare type of non-Hodgkin's lymphoma found in adults. Since its discovery in the 1960's, most of the available information regarding this disease entity has been collected through individual case reports, with an estimated incidence of less than one person per million. Few reports cover large series of cases. A study of 38 Western patients diagnosed with intravascular lymphoma was made in 2004 in relation to clinical presentation and natural history.[1] The largest study to date reported a series of 96 patients diagnosed with IVLBCL in Japan, addressing clinical profiles, outcomes and prognostic factors.[2]

IVLBCL is characterized by the selective growth of neoplastic cells within the blood vessel lumina.[3] It has been dubbed "the oncologist's great imitator" due to its protean manifestation. The disease manifests as fever of unknown origin and is frequently diagnosed post-mortem due to its aggressive clinical course.[4] Neoplastic cells are not usually seen in the peripheral blood smear, but laboratory findings of anemia, elevated serum lactate dehydrogenase, thrombocytopenia and leukopenia should raise a suspicion for IVLBCL.[5] Increased awareness of this disease entity, for both clinicians and pathologists, may clinch the diagnosis in the future.

CASE

This is a case of a 64-year-old female who was admitted due to a three-week history of intermittent fever. She had no associated symptoms such as cough or colds. The past medical, personal and social history were unremarkable. Physical examination revealed palpable cervical lymph nodes and lower gastrointestinal bleeding secondary to hemorrhoids; the rest were unremarkable. Several diagnostic work-ups were done to determine the etiology of the fever. Complete blood counts show anemia (8.8 g/dL) and thrombocytopenia (71-137 x 10^9/L). She had normal levels of white blood cells with predominance of segmenters. Blood culture was negative. CT scan of the chest reveal non-specific pulmonary nodules in the right lung and cardiomegaly. To rule out a malignant etiology of gastrointestinal bleeding, abdominal

CT scan was also done which showed dilated and tortuous blood vessels on both liver lobes and esophageal varices, suggestive of portal hypertension. These findings, however, could not explain the cause of fever. Gastroscopy and colonoscopy findings were non-contributory. During the hospital stay, she developed cough associated with difficulty of breathing. Chest radiograph showed streaky hazy infiltrates in both bases for which she was managed as a case of hospital-acquired pneumonia and started on antibiotics. Work-up for autoimmune disorders (ANA, C3 and RF) were negative. At this time, a hematolymphoid malignancy was being considered. Bone marrow biopsy findings showed a variably cellular marrow (70-90%) with mild panhyperplasia and slight lymphocytosis. No definite lymphoid aggregates or granulomatous infiltrates are seen. A correlation with clinical findings was recommended. Serum lactate dehydrogenase was requested and was found to be elevated at 3349 U/l. On her 3rd hospital week, the patient had episodes of waxing and waning of sensorium associated with a rigid neck. Cranial MRI and CSF studies were done to rule out meningitis which all turned out to be unremarkable. With the patient's history of prolonged fever without an infectious focus and a significantly elevated LDH (6000 U/l), consideration at that time was leaning towards intravascular lymphoma. Further work-up was done at the interim, but bone marrow biopsy results were still negative. The condition of the patient gradually deteriorated and she succumbed on the 24th hospital day.

Autopsy was performed 17 hours after the patient's demise. On external examination, she had multiple flat, irregular, erythematous lesions distributed on her left shoulder, right forearm, abdomen and lower extremities (Figure 1). There were no palpable masses or lymphadenopathies. Cardiomegaly (430 grams; normal 179-385 grams) and splenomegaly (265 grams; normal 55-195 grams) was noted after weighing all the organs. The right and left ventricular walls were hypertrophic. Both lungs had boggy, subcrepitant parenchyma. The liver was not enlarged. Although smooth upon palpation, the parenchyma appeared to have small nodules which led us to assume the patient has micronodular cirrhosis which would explain her signs of portal hypertension. Macroscopic examination of the gastrointestinal tract showed esophageal varices and gastric ulcers and a colonic polyp. Infarcts were noted the base of the pons, left cerebellar peduncle and left internal capsule (Figure 2). The endocrine and genitourinary organs were unremarkable. Representative sections were taken and submitted for histopathological examination.

Microscopic examination reveal aggregates of atypical lymphoid cells in the small to medium-sized blood vessels and sinusoids of the following organs: lungs, liver, spleen, kidneys, uterus, cervix, brain, heart and thyroid. These cells have increased nuclear-to-cytoplasmic ratio, irregular nuclei, some with prominent nucleoli. Immunohistochemical staining revealed positivity for B-cell marker CD20, and negative for CD3, CD10 and CD30. Post-mortem bone marrow sample was noted to be hypercellular (70-80%) with trilineage maturation. Immunohistochemical staining with CD20, however, was negative. The findings of intravascular neoplastic proliferation of atypical lymphocytes in several organs, absence of lymph node involvement, and positivity for CD20 led us to the diagnosis of intravascular diffuse large B-cell lymphoma (Figure 3).

DISCUSSION

Intravascular large B-cell lymphoma (IVLBCL) is a rare form of extranodal diffuse LBCL characterized by proliferation of lymphoma cells within the lumina of vessels, particularly capillaries, with exception of large arteries or veins.[3] It was previously called angiotropic large cell lymphoma. This entity is typically found in adults, with a median age of 67 years, and is found to have equal distribution among both sexes. It has an aggressive clinical course, usually widely disseminated and may present virtually on any organ. Lymph nodes are usually spared. Intravascular growth pattern has been hypothesized to be secondary to a defect in homing receptors on the neoplastic cells such as lack of CD29 and CD54 (ICAM-1) adhesion β-molecules.[4] Fever of unknown origin is a prominent sign and is seen in approximately 45% of cases.[5] Laboratory findings are not specific but should raise IVLBCL suspicion, and these include anemia, elevated serum lactate dehydrogenase, elevated erythrocyte sedimentation rate, thrombocytopenia, leukopenia and hypoalbuminemia. Majority of the studies have shown neoplastic cells to be absent in peripheral blood examination.[6] Western and Asian variants have been identified based on clinical presentation. Patients from Western countries display a high frequency of CNS and skin involvement, while those from Asian countries show hemophagocytic syndrome, bone marrow involvement, fever, hepatosplenomegaly and thrombocytopenia.[7] In a clinicopathological study of diagnosed IVLBCL cases in China, 8 out of 13 patients displayed bone marrow involvement, supported by immunohistochemistry. They concluded that a morphological "sinusoidal pattern" is essential to the diagnosis

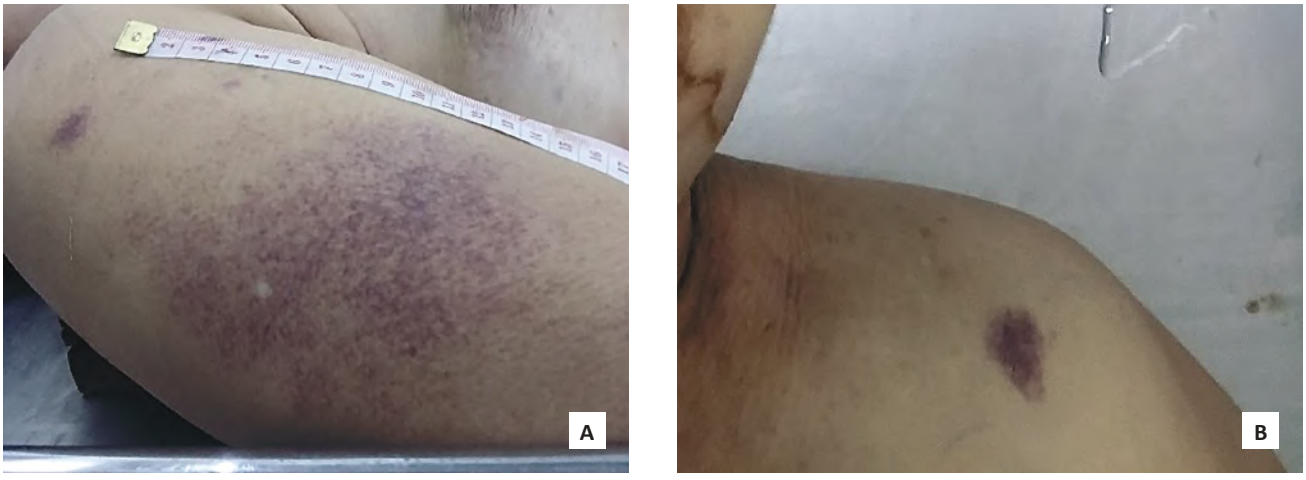


Figure 1. Skin lesions. **(A)** Flat, irregular, red to violaceous, rash-like lesion on the right upper arm; **(B)** Flat skin lesion on the left shoulder.

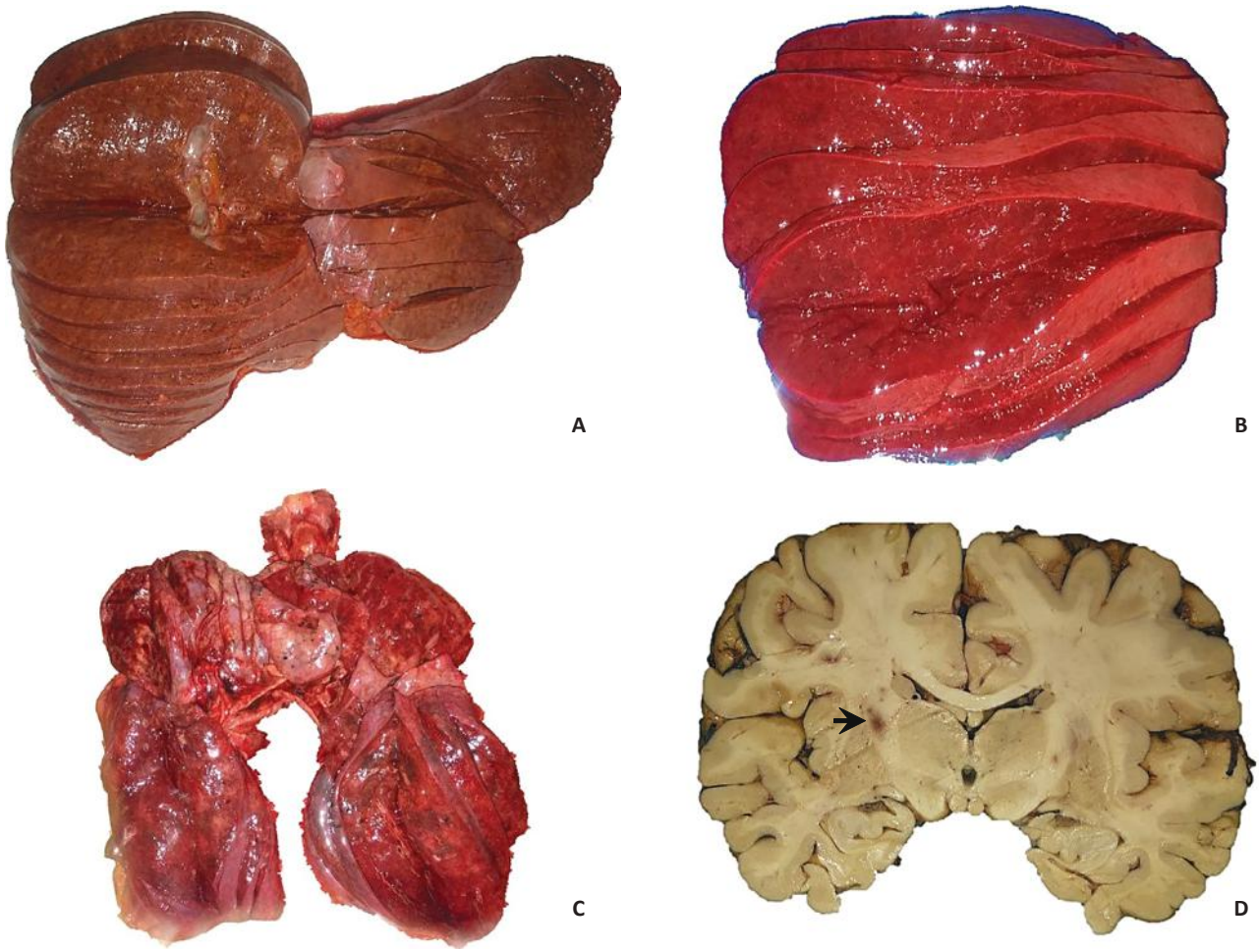


Figure 2. Gross appearance of the organs. Cut sections of the liver **(A)** and spleen **(B)**. The right and left lungs (C) have red to brown, soft, boggy, sub-crepitant parenchyma; air spaces are not enlarged. Focal, dark brown, infarct-like lesions are noted on the brain parenchyma. The arrow points to the lesion seen in the internal capsule **(D)**.

of IVLBCL in a bone marrow biopsy.[8] In our case, however, bone marrow biopsy results were negative.

Hepatic injury was clinically evident in this case. In the wards, she was noted to have elevated serum ammonia, SGPT, alkaline phosphatase and bilirubin levels. Esophageal varices and hemorrhoids support the diagnosis of portal hypertension on Doppler ultrasound. Consequently, the increased portal blood flow led to enlargement of the spleen. Another IVLBCL case with the same manifestation was reported in a 62-year-old male,[9] but no thorough discussion was made regarding the portal hypertension. Microscopically, the liver of our patient was not cirrhotic. Histologic examination showed diffuse sinusoidal infiltration of the liver parenchyma. Lymphoma has been cited as a cause neoplastic occlusion of the portal vein.[10] Though IVLBCL is said to be restricted to the smallest of blood vessels, one case report mentioned the embolization of lymphoma cells in the hepatic portal vein, splenic vein and mesenteric vein.[11]

Cutaneous involvement varies in appearance and may present as painful indurated erythematous eruptions, poorly circumscribed violaceous plaques, to palpable purpura or small red palpable spots. Unfortunately for our case, a skin biopsy was not carried out on post-mortem examination. One study recommends performing skin biopsy irrespective of the presence or absence of skin lesions in patients who are suspected to have IVLBCL after they have concluded its high sensitivity compared to bone marrow biopsy.[12] Symptoms secondary to CNS involvement are common and varied and may range from sensory and motor deficits, altered conscious state, paresthesias to seizures.[13]

Quite unusual in this case is the presence of the neoplastic cells confined in the small blood vessels of the uterus and cervix. Although it has been frequently mentioned in different studies that IVLBCL can virtually involve any organ, the infiltration of the blood vessels in the gynecologic tract is rarely noted. At least 3 case reports describe the diagnosis of IVLBCL in the uterus, cervix and ovaries[14-16]—all of which noted the absence of a defined mass macroscopically, and confirming the diagnosis on histopathological examination.

Patients with IVLBCL have a poor prognosis, partly due to its protean manifestation and diagnostic delays. With its high overall mortality rate, more than half of the patients are diagnosed post-mortem.[17] It responds poorly to chemotherapy and neither clinical types nor clinical parameters can predict survival, apart from better prognosis for cases with disease limited to the skin.

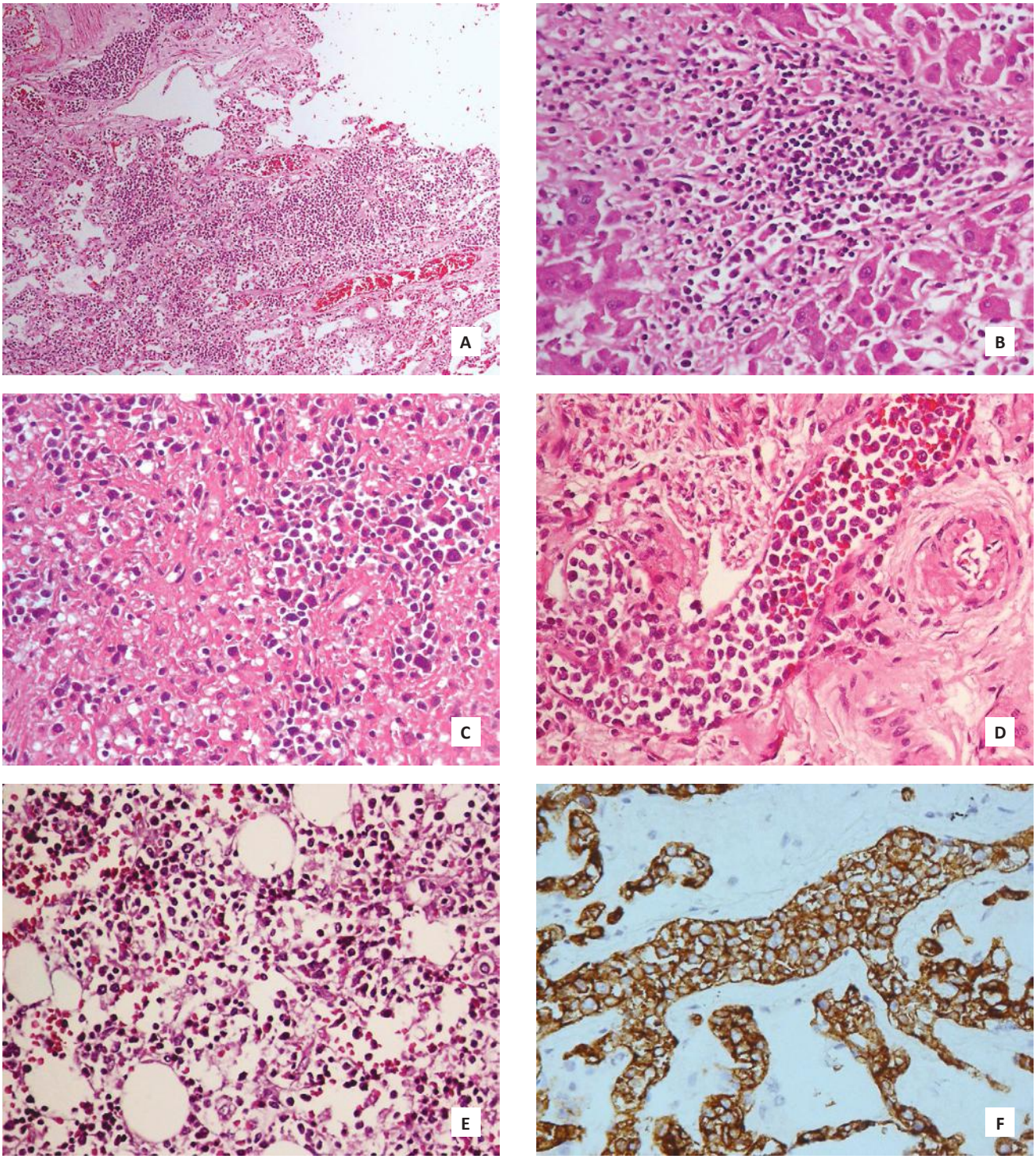


Figure 3. Intravascular large cell B-lymphoma. Proliferation of neoplastic lymphoid cells within the lumen of small to medium-sized blood vessels are noted in the following organs: **(A)** Lungs, H&E, 10x **(B)** Liver, H&E, 40x **(C)** Spleen, H&E, 40x **(D)** Uterus, H&E, 40x. **(E)** Hypercellular bone marrow with trilineage maturation. Immunohistochemistry with CD3, CD20 and CD30 were all negative. **(F)** Neoplastic cells found in the blood vessels of the lungs show strong, cytoplasmic reactivity to CD20, 40x. The same immunohistochemistry pattern was noted in the liver, spleen, kidneys, uterus, cervix, brain, heart and thyroid.

CONCLUSION

Despite numerous efforts, a definitive diagnosis of B-cell lymphoma was not established before the patient's demise. In an adult patient who presents with fever of unknown origin associated with anemia, elevated serum lactate dehydrogenase, thrombocytopenia and leukopenia, altered hepatic, renal or thyroid function tests, a differential diagnosis of IVLBCL should be considered. A bone marrow or skin biopsy may be helpful. However, should the case present as a clinical dilemma such that diagnosis cannot be rendered prior to the patient's demise, postmortem examination is recommended to further increase the awareness for this disease entity and to better understand its pathogenesis.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Ferreri A, Campo E, Seymour JF, et al. Intravascular lymphoma: clinical presentation, natural history, management and prognostic factors in a series of 38 cases, with special emphasis on the 'cutaneous variant'. Br J Haematol. 2004;127(2):173-83. PMID: 15461623. https://doi.org/10.1111/j.1365-2141.2004.05177.x.
2. Murase T, Yamaguchi M, Suzuki R, et al. Intravascular large B-cell lymphoma (IVLBCL): a clinicopathological study of 96 cases with special reference to the immunophenotypic heterogeneity of CD5. Blood. 2007;109(2):478-85. PMID: 16985183. https://doi.org/10.1182/blood-2006-01-021253.
3. Nakamura S, Ponzoni M, Campo E. Intravascular large B-cell lymphoma. In S. Swerdlow, E. Campo, Harris NL, editors. WHO classification of tumours of haematopoeitic and lymphoid tissues, 4th ed. Lyon: IARC. 2008, pp.252-3.
4. Ponzoni M, Arrigoni G, Gould VE, et al. Lack of CD29 (beta1 integrin) and CD 54 (ICAM-1) adhesion molecules in intravascular lymphomatosis. Hum Pathol. 2000;31(2):220-6. PMID: 10685637.
5. Holmes NE, Gordon CL, Lightfoot N, et al. Intravascular large B cell lymphoma: an elusive cause of pyrexia of unknown origin diagnosed postmortem. Clin Infect Dis. 2010; 51(9): e61-4. PMID: 20868278. https://doi.org/10.1086/656684.
6. Zuckerman D, Seliem R, Hochberg E. Intravascular lymphoma: the oncologist's "great imitator". Oncologist. 2006;11(5):496-502. PMID: 16720850. https://doi.org/10.1634/theoncologist.11-5-496.
7. Ponzoni M, Ferreri AJ, Campo E, et al. Definition, diagnosis, and management of intravascular large B-cell lymphoma: proposals and perspectives from an international consensus meeting. J Clin Oncol. 2007;25(21):3168-73. PMID: 17577023. https://doi.org/10.1200/JCO.2006.08.2313.
8. Wang J, Ding W, Gao L, et al. High frequency of bone marrow involvement in intravascular large B-cell lymphoma: a clinicopathological study of 13 cases in China. Int J Surg Pathol. 2016;25(2):118-26. PMID: 27553679. https://doi.org/10.1177/1066896916665203.
9. Fiegl M, Greil R, Pechlaner C, Krugmann J, Dirnhofer S. Intravascular large B cell lymphoma with a fulminant clinical course: a case report with definite diagnosis post mortem. Ann Oncol. 2002;13(9):1503-6. PMID: 12196378.
10. Khanna R, Sarin S. Non-cirrhotic portal hypertension--diagnosis and management. J Hepatol. 2014;60(2): 421-41. PMID: 23978714. https://doi.org/10.1016/j.jhep.2013.08.013.
11. Yasuda, H, Ando J, Matsumoto T, et al. Intravascular large B cell lymphoma with hepatic portal vein, splenic vein and mesenteric vein tumour embolism. Histopathology. 2010;57(4):648-50. PMID: 20955393. https://doi.org/10.1111/j.1365-2559.2010.03660.x.
12. Matsue K, Asada N, Odawara J, et al. Random skin biopsy and bone marrow biopsy for diagnosis of intravascular large B cell lymphoma. Ann Hematol. 2011;90(4):417-21. PMID: 20957365. https://doi.org/10.1007/s00277-010-1101-3.
13. Orwat DE, Batalis NI. Intravascular large B-cell lymphoma. Arch Pathol Lab Med. 2012;136(3):333-8. PMID: 22372911. https://doi.org/10.5858/arpa.2010-0747-RS.
14. Sur M, Ross C, Moens F. Intravascular large B-cell lymphoma of the uterus: a diagnostic challenge. Int J Gynecol Pathol. 2005;24(2):201-3. PMID: 15782078.
15. Shigematsu Y, Matsuura M, Tsuyama N, et al. Intravascular large B-cell lymphoma of the bilateral ovaries and uterus in an asymptomatic patient with a t(11;22)(q23;q11) constitutional translocation. Intern Med. 2016;55(21):3169-74. PMID: 27803414. PMCID: PMC5140869. https://doi.org/ 10.2169/internalmedicine.55.6578.
16. Fournier M, Hourseau M, Chis C, Luton D, Koskas M. Uterine presentation of an intravascular large B-cell lymphoma diagnosed by PET/CT and histology. Case Rep Clin Pathol. 2014;1(2):105-8. https://doi.org/10.5430/crcp.v1n2p105.
17. Wahie S, Dayala S, Husain A, Summerfield G, Hervey V, Langtry JA. Cutaneous features of intravascular lymphoma. Clin Exp Dermatol. 2011;36(3):288–91. PMID: 21418271. https://doi.org/10.1111/j.1365-2230.2010.03934.x.

External Quality Assessment Scheme for Transfusion Transmissible Infections among Blood Service Facilities in the Philippines, 2016

Kenneth Aristotle Punzalan, Rhoda Yu, Iza Mae Chamen

Research Institute for Tropical Medicine – Department of Health, Philippines

ABSTRACT

The External Quality Assessment Scheme for Transfusion Transmissible Infections in the Philippines aims to raise the standards of quality testing for infectious diseases in blood units.

The National Blood Program lists more than 200 Blood Service Facilities (BSF) in the country in which 162 participated in the 2016 EQAS test event. These participants were given an EQAS panel composed the HVHT4320 serology program and MLRA415 malaria program. The panels should be treated by the participants as routine donor samples to simulate the actual laboratory process which allows the NRL and the participant to check and validate the entire blood unit screening process.

The results were submitted via an online informatics system and were analyzed by One World Accuracy Canada using the ISO 13528:2008 Robust Statistics method (Huber's Method) to identify outliers. Qualitative results were evaluated and compared with the reference results of the NRL to which non-concordance would mark their results aberrant. The results of the test event showed a number of participants having aberrant results due to either random or systematic errors.

Data gathered from this EQAS test event are used to improve the processes of the blood service facility to ensure quality testing.

Key words: quality assurance, blood donor serology, transfusion transmissible infections, proficiency testing

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 30 June 2017.
Accepted: 10 August 2017.
Published online first: 15 August 2017.
https://doi.org/10.21141/PJP.2017.015

Corresponding author: Kenneth Aristotle P. Punzalan, RMT
E-mail: kenn.punzalan@gmail.com, tti.nrl@gmail.com

INTRODUCTION

External Quality Assessment is a crucial aspect of quality assurance in medical laboratories. The results generated from each test event continuously reflect the analytical quality of the measurements performed by the participating laboratory and the performance can also be compared with other laboratories using the same instrument or method.[1]

The External Quality Assessment Scheme (EQAS) for Transfusion Transmissible Infections (TTI) in the Philippines is a mandatory requirement for licensing of Blood Service Facilities whose category are Blood Centers and/or Blood Bank with Additional Functions[2] that aims to raise the standards on quality testing of blood units and assess each phase of testing to determine inter-laboratory comparison.

This activity intends to assess the quality of blood unit testing of blood service facilities in the Philippines for the EQAS 2016 test event.

METHODOLOGY

Panel Composition

The TTI EQAS test event consists of two panels, the HVHT4320 for blood donor serology, and the MLRA415 for malaria slide microscopy. The HVHT4320 consists of twenty (20) pooled plasma samples obtained from blood donors from different regions of the country. Each pooled sample was prepared by mixing similar volumes of at least two samples that had similar antibody and antigen profiles. All samples were subjected to filtration prior

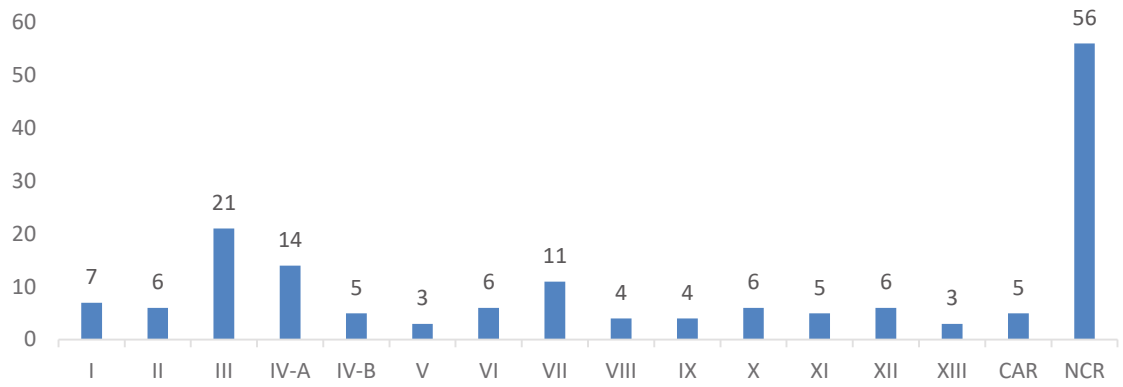


Figure 1. Regional distribution of participants.

to aliquoting. The samples were aliquoted and their homogeneity confirmed. The serology profile for HIV, HBV, HCV, Syphilis of each sample were identified using a chemiluminescence assay (ChLIA), enzyme immunoassay (EIA), Rapid Plasma Reagin (RPR), Particle Agglutination (PA) and Western Blot (WB).

Program code MLRA415 consists of five (5) blood smears. The samples were obtained from Malaria patients in Palawan and prepared by the NRL for Malaria and other Parasites of the Research Institute for Tropical Medicine.

Participants

The Multimarker Blood Serology EQAS panel ID HVHT4320 and Malaria Microscopy EQAS Panel ID MLRA415 were distributed to 162 participants nationwide. These participants enrolled for the EQAS 2016 test event with a corresponding registration fee to cover expenses for the test event.

Majority of the participants were private institutions (43%) followed closely by government institutions (41%) and the remainder are from the Philippine Red Cross (16%). Figure 1 shows the distribution of participants by region.

Data Analysis

ISO 13528:2005 Robust Statistics method (Huber's Method) was used to identify outlying results (numerical test results found to be statistically different from other test results reported by participants that tested the same sample in the same assay) for the created peer groups. A peer group is defined as a set of laboratories that utilize the same test format and assay test kit for screening TTI. The said method uses the mean as an estimator and outlying test results were removed from statistical calculation. Qualitative results of the BSF were compared with the qualitative reference results of the NRL Discrepancy between the two results would mark a result aberrant.

RESULTS AND DISCUSSION

Majority of the participants used the ChLIA platform (48.77%) in testing the panels followed by EIA (29.63%). 6.79% used a combination of ChLIA and EIA, 1.85% used rapid test kits alone, and 12.96% used a combination of either ChLIA, EIA, Rapid Test Kits (RDT), Rapid Plasma Reagin (RPR), and Particle Agglutination (PA).

4.94% of the total participants had data entry errors or clerical errors (e.g. reactive test results were interpreted as negative or vice versa).

Of the 162 participants, 29.01% reported aberrant results for the HVHT4320 serology panel. A total of 13,374 results were reported by the participants and 91 (0.68%) were marked as aberrant. From the aberrant results, 47 (51.65%) were reported as false positive, 27 (29.67%) were reported as false negative, and 17 (18.68%) were reported as inconclusive. From the 27 false negative results, 11 (40.74%) were due to clerical errors.

Distribution of aberrant results by platform and analyte for the initial panel is shown in Table 1. These aberrant results were either due to data entry errors, sample mix-up or sample carry-over (particularly where an instrument was used in assay set-up).

The following criteria must be met for a participant to be classified as an unsatisfactory performer in the HVHT4320 initial panel: (a) at least one false negative result; (b) at least twenty percent (20%) false positive results. In accordance with these criteria, corresponding participants were given an investigation checklist to assist them in identifying errors and make the necessary corrective actions and/or troubleshooting methods. A 2nd set of the HVHT4320 panel were given to the participants for retesting if the identified unsatisfactory performance was due to a testing error. Participants with aberrant results due to transcription errors were only given an investigation/troubleshooting checklist and a

Table 1. Number of aberrant results per transfusion transmissible infections testing platform (HVHT4320 1st panel), EQAS 2017

Platform	HIV			HBV			HCV			SYP			TOTAL ABERRANT
	FN	INC	FP	FN	INC	FP	FN	INC	FP	FN	INC	FP	
ChLIA	1 (1.10%)	0 (0.00%)	4 (4.40%)	7 (7.69%)	0 (0.00%)	17 (18.68%)	5 (5.49%)	3 (3.30%)	4 (4.40%)	1 (1.10%)	2 (2.20%)	0 (0.00%)	44 (48.35%)
EIA	1 (1.10%)	3 (3.30%)	11 (12.09%)	1 (1.10%)	1 (1.10%)	4 (4.40%)	3 (3.30%)	0 (0.00%)	3 (3.30%)	0 (0.00%)	0 (0.00%)	4 (4.40%)	31 (34.07%)
RDT	0 (0.00%)	4 (4.40%)	0 (0.00%)	6 (6.59%)	0 (0.00%)	0 (0.00%)	1 (1.10%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	11 (12.09%)
RPR	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1 (1.10%)	4 (4.40%)	0 (0.00%)	5 (5.49%)
TOTAL	2 (2.20%)	7 (7.69%)	15 (16.48%)	14 (15.38%)	1 (1.10%)	21 (23.08%)	9 (9.89%)	3 (3.30%)	7 (7.69%)	2 (2.20%)	6 (6.59%)	4 (4.40%)	91 (100.00%)

Legend: FN – False Negative, FP – False Positive, INC – Inconclusive, HIV – Human Immunodeficiency Virus, HBV – Hepatitis B Virus, HCV – Hepatitis C Virus, SYP – Syphilis

written recommendation. Eight (8) participants were identified with transcription errors which they have recognized in the given investigation checklist. Ten (10) participants were given a second set of samples wherein one had reported a false negative result.

For the MLRA415 panel, 33% of the participants reported aberrant results with 8.64% reporting false positive results and 30.25% reporting false negative results.

Figure 2 shows the distribution of grades of the participants. They have been evaluated and graded as follows:

- Excellent – 100% acceptable results on the initial panel (all final results were correctly identified in comparison with the reference results);
- Very Satisfactory – Less than 100% acceptable results on the initial panel without being given a second panel for retesting.
- Satisfactory – 100% acceptable results on retesting of the second panel; or had an aberrant result in the initial panel due to a clerical error, given that the participant was able to identify this error through the EQAS investigation checklist.
- Poor – Participant did not follow minimum requirements of testing as per DOH Circular No. 2013-0132 or less than 100% acceptable results on retesting of the second panel; or had an aberrant result in the initial panel due to a clerical error which the participant had failed to identify in the EQAS investigation checklist.

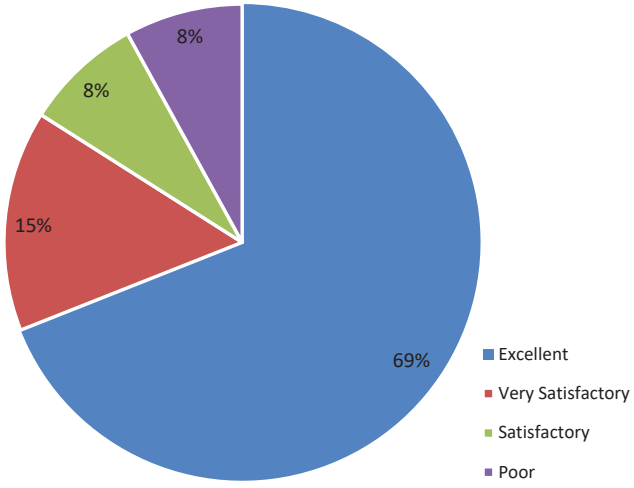


Figure 2. Distribution of grades for the EQAS 2016 test event.

CONCLUSION

The TTI EQAS is a valuable management tool aimed to improve the efficiency and service of a laboratory. While this event has shown a number of participants failing, the results should be used as an opportunity to compare their activities and remodel their current practices based on what they would learn. A strong commitment from top-level managers of these participants is essential to improve these processes.

RECOMMENDATION

The participating laboratory should be responsible in reviewing their EQAS report and in discussing it to the people involved in the process since this is an opportunity for improvement by way of a corrective action. The analyzed data can improve the quality of results from the participants as this can be used to as evidence to introduce or improve the quality assurance of the laboratory.[3] An increase in the number of EQAS test events within a year would be of value in the improvement of the BSF processes.

ACKNOWLEDGMENTS

The authors thank the TTI-NRL staff, Dr. Catherine Masangkay and Dr. Socorro Lupisan of the Research Institute for Tropical Medicine (RITM), the Health Facility Development Bureau, the Health Facility Services and Regulatory Bureau, and the National Voluntary Blood Services Program of the Department of Health, the National Council for Blood Services–Technical Working Group, the Department of Parasitology (RITM), the Philippine Red Cross – National Blood Center (Port Area), the Asian Hospital and Medical Center, and OneWorld Accuracy – Canada.

We also extend our thanks to all the participating Blood Service Facilities for their support.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Kristensen GB. Flowchart for Handling Deviating Results from External Quality Assessment. Int J Health Care Qual Assur. 2010; 20(1):37.
2. Department of Health. DOH Department Memorandum No. 2009-0086B: Amendment to Department Memorandum No. 2009-0086-A entitled, "Implementation of external quality assessment program as regulatory requirement for licensing of clinical laboratories." 8 September 2014. Retrieved from http://ttinrl. com/wp-content/uploads/2014/11/DM-2009-0086-B.pdf.
3. World Health Organization. WHO manual for organizing a national external quality assessment programme for health laboratories and other testing sites. Geneva, Switzerland, 2016. Available from: http://www.who.int/hiv/pub/toolkits/manual-external-quality-assessment-testing/en/.

Osteolipoma: A Rare Variant of the Common Lipoma

Emilio Villanueva III[1] and Ariel Vergel de Dios[2]

[1]*Department of Laboratories, University of the Philippines-Philippine General Hospital*
[2]*Department of Pathology, University of the Philippines-College of Medicine*

Key words: osteolipoma, lipoma, variant

A 78-year-old female consulted for a ten-year gradually enlarging, right medial thigh mass, measuring 40 x 40 x 30 cm at time of consult. Physical examination shows right lower extremity external rotation, left lower extremity genu varum, and difficulty in walking due to mass effect. No imaging procedures was done; the patient was immediately scheduled for surgery after pre-operative clearance. Excision of the thigh mass was performed to relieve the patient of the mass effect. Intraoperative finding was a fibro-fatty mass located in the medial compartment of the thigh. There was no attachment to the femur nor the major blood vessels. The mass was submitted for histopathologic examination.

Gross examination shows yellow to cream/yellow, ovoid, fibrofatty to firm tissue with cream/white to cream/yellow smooth, glistening cut sections, with patchy areas of calcifications and fibrosis. Histopathologic examination shows mature adipose tissue with admixed mature lamellar bone (Figure 1) and areas with hyaline cartilage undergoing ossification (Figure 2). The adipocytes are monotonous and monomorphic. There is no nuclear atypia, lipoblasts, mitotic activity, nor necrosis seen. The case was signed out as osteolipoma.

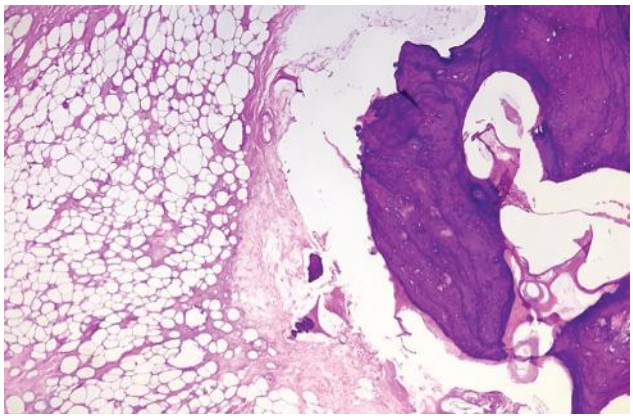


Figure 1. Mature adipose tissue with admixed mature lamellar bone (Hematoxylin and Eosin at 40x).

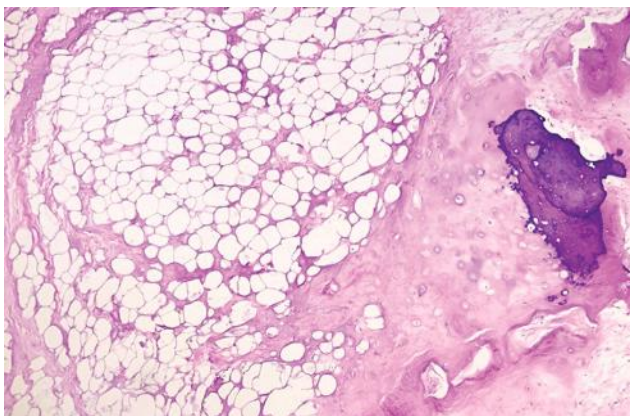


Figure 2. Mature adipose tissue with hyaline cartilage undergoing ossification (Hematoxylin and Eosin at 40x).

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 12 July 2017.
Accepted: 29 July 2017.
Published online first:
https://doi.org/10.21141/PJP.2017.016

Corresponding author: Emilio Q. Villanueva III, MD, RMT, MT(ASCPi)
E-mail: drevilthird@gmail.com

Lipomas are the most common benign soft tissue tumor occurring in subcutaneous tissues. Most patients are adults in 30's to 40's. It is comprised of mature adipocytes without atypia.[1,2] Variants of lipomas exist, having other mesenchymal components such as fibrous, smooth muscle, myeloid, chondral, vascular, or osseous tissue present admixed with the adipocytes. The nomenclature of the different variants depends on the type of tissue admixed with the adipocyte such as fibrolipoma, leiomyolipoma, myelolipoma, chondrolipoma, angiolipoma, and osteolipoma, respectively.[2] The rare variant osteolipoma is composed of mature lamellar bone interspersed within the adipose tissue.[3] Microscopically, they appear as predominantly mature adipose tissues with irregularly distributed mature lamellar bone with areas of ossification. This is the main morphologic criteria of the diagnosis.[4]

The principal pathologic differential diagnosis of a deep-seated lipoma is a well-differentiated liposarcoma. It is a deep-seated tumor common in adults above 50 years old. Morphologically, it is also composed of mature adipocytes. It is differentiated by the presence of scattered hyperchromatic nuclei, mostly situated within the fibrous septa.[5]

There are two main theories regarding the pathogenesis of osteolipoma: first, they may be directly derived from multipotent mesenchymal cells;[2] another, they may arise after repetitive trauma, ischemia, or metabolic changes initiating osseous metaplasia. The adipose tissue component strengthens this osteoblastic activity.[4,6]

Osteolipomas have good prognosis similar with simple lipoma. Surgical excision is the recommended treatment.[1,4]

REFERENCES

1. Cheng S, Lu SC, Zhang B, Xue Z, Wang HW. Rare massive osteolipoma in the upper part of the knee in a young adult. Orthopedics. 2012;35(9):e1434-7. PMID: 22955415. https://doi.org/10.3928/01477447-20120822-35.
2. Demiralp B, Alderete JF, Kose O, Ozcan A, Cicek I, Basbozkurt M. Osteolipoma independent of bone tissue: a case report. Cases J. 2009;2:8711. PMID: 19918398. PMCID: PMC2769468. https://doi.org/10.4076/1757-1626-2-8711.
3. Fritchie KJ, Renner JB, Rao KW, Esther RJ. Osteolipoma: radiological, pathological, and cytogenetic analysis of three cases. Skeletal Radiol. 2012;41(2):237-44. PMID: 21822651. https://doi.org/10.1007/s00256-011-1241-0.
4. Electricwala AJ, Panchwagh Y, Electricwala JT. Giant osteolipoma fixed to the greater trochanter of the femur in a seventy-year-old elderly woman. Cureus. 2017;9(2):e1036. PMID: 28357168. PMCID: PMC5356992. https://doi.org/10.7759/cureus.1036.
5. Fisher C, Montgomery EA, Thway K. Biopsy interpretation of soft tissue tumors. Philadelphia: Lippincott Williams & Wilkins, 2011.
6. Heffernan EJ, Lefaivre K, Munk PL, Nielsen TO, Masri BA. Ossifying lipoma of the thigh. Br J Radiol. 2008;81(1968):e207-10. PMID: 18628326. https://doi.org/10.1259/bjr/38805072.

Contrast-Enhanced Spectral Mammography: A Radiologic-Pathologic Perspective of a Novel Functional Imaging Modality for Breast Cancer

Ma. Theresa Buenaflor[1] and Francis Moria[2]

[1]*Health Cube Advanced Medical Imaging Unit-San Juan City, Philippines*
[2]*College of Medicine, St. Luke's Medical Center-Quezon City, Philippines*

ABSTRACT

Contrast-enhanced Spectral Mammography (CESM) is an emerging and promising functional imaging modality that tries to address the paucity of physiologic-based tumor imaging for the detection of breast cancer.

This article describes two cases of women with non-dense and dense breasts presenting with clinically palpable breast masses and the depiction of breast cancer utilizing Contrast-enhanced Spectral Mammography.

Key words: Contrast-Enhanced Spectral Mammography (CESM), Digital Breast Tomosynthesis, Magnetic Resonance Imaging, Low energy, Subtracted Image, Full-Field Digital Mammography (FFDM)

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 29 September 2017.
Accepted: 2 November 2017.
Published online first: 15 November 2017.
https://doi.org/10.21141/PJP.2017.017

Corresponding author: Ma. Theresa S. Buenaflor, MD, FPCR
E-mail: buenates2@gmail.com

INTRODUCTION

Mammography is the only breast imaging modality with a demonstrated ability to reduce mortality.[1] A review by Myers et al., confirmed the findings of several published studies that screening mammography in women aged 40-79 reduces breast cancer mortality rates by 20%-50%, with extent of benefit varying by age, as well as study design (RCT vs. observational).[2] However, mammography has a population-based sensitivity of only approximately 80%[1] which is further corroborated by the findings of Carney et al., that showed that with increasing breast density, the sensitivity of mammography decreases to 62% in women with dense breasts.[3]

Breast density refers to the proportion of glandular and fibrous breast tissue to the amount of fatty tissues in a woman's breast. It has been shown that women who have high density breasts are 4-5x more likely to get breast cancer than women with low breast density.[4,5] It is also statistically significantly greater among Asian women than among African American and white women.[6]

Contrast-Enhanced Spectral Mammography (CESM)

Contrast-enhanced Spectral Mammography (CESM) is a novel imaging modality that demonstrates the physiologic uptake of contrast by breast cancer. The depiction of breast cancers using contrast media is based on the biologic principle of the rapid formation of tumoral microvessels that render malignancy-associated vessels more permeable to contrast agent than normal tissue, resulting in tumor enhancement.[7]

It has been proposed by Chang et al., that the use of a standard iodinated CT contrast agent and x-ray imaging might also give functional information with a preferential uptake in breast cancers.[8] An early study done among 26 subjects in 2003 by Lewin et al., of the University of Colorado utilizing dual-energy contrast-enhanced mammography showed tumor enhancement in 13 of the subjects that had subsequent biopsy-proven invasive cancers.[9]

Contrast-enhanced Spectral Mammography was introduced in Europe in June 2010 and received FDA approval in the United

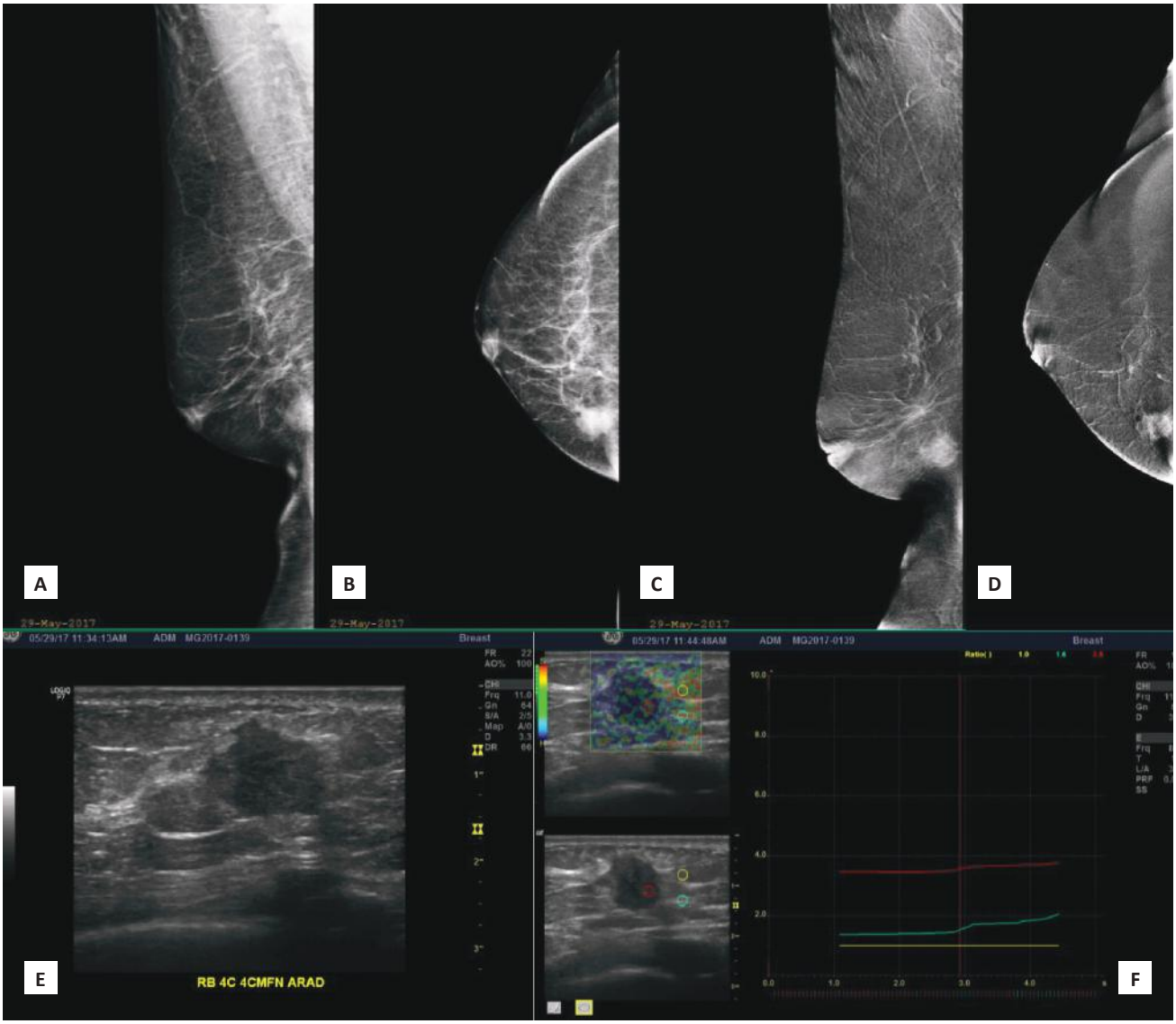


Figure 1. A 63-year-old post-menopausal female with a newly palpable right breast mass with no family history of breast cancer. **(A, B)** Low-energy (LE) Mediolateral Oblique (MLO) and Craniocaudal (CC) views of the right breast, which has a fatty breast composition, showing an irregular mass of high density at the lower inner quadrant. **(C, D)** Subtracted images (SI) in MLO and CC projections show avid enhancement of the right lower inner quadrant breast mass. No other abnormal enhancing lesions are demonstrated. The background parenchyma also shows no enhancement. **(E)** Ultrasound correlate of the said mass shows an irregular markedly hypoechoic solid mass. **(F)** Elastography (which is a measure of tissue stiffness) shows the mass to be significantly hard compared to the adjacent fat and glandular tissues.

States on October 2011. The first center in the UK to acquire the technology was Nottingham Breast Unit and in the United States, early adopters include Memorial Sloan-Kettering Cancer Center in 2010. In Southeast Asia, the early proponents of CESM are Taiwan and Thailand in 2012.

The advantages of using CESM are that it is similar in diagnostic performance with Magnetic Resonance Imaging. Two studies have shown the similarity of Contrast-enhanced Spectral Mammography to MRI. Jochelson et al., found that CESM and MRI have equal sensitivity (96%) while Fallenberg et al., found CESM to have 100% sensitivity compared with 97% sensitivity for MRI.[10,11] This was further corroborated by Lee-Felker et al., who also had similar sensitivity of CESM to MRI (94% vs. 99%) as well as having a significantly higher PPV (Positive Predictive Value) of 93% compared to 60% of MRI.[12]

It can be used in patients with contraindications to doing MRI such as claustrophobia and/or averse to gadolinium contrast.

Furthermore, a recent study by Patel et al., showed that CESM had a reduced exam time of 7-10 minutes compared to MRI with 30-60 minutes as well as reduced staff time of 25 minutes compared to 60 minutes.[13]

The disadvantages include its contraindication for use in patients with abnormal renal function or if they have a known reaction to iodine contrast. Furthermore, it is not advised for pregnant or lactating women or those who are diagnosed with hyperthyroidism.

How is it performed?

Before a CESM exam is initiated, a thorough history is elicited from the patient with emphasis on allergy history and previous

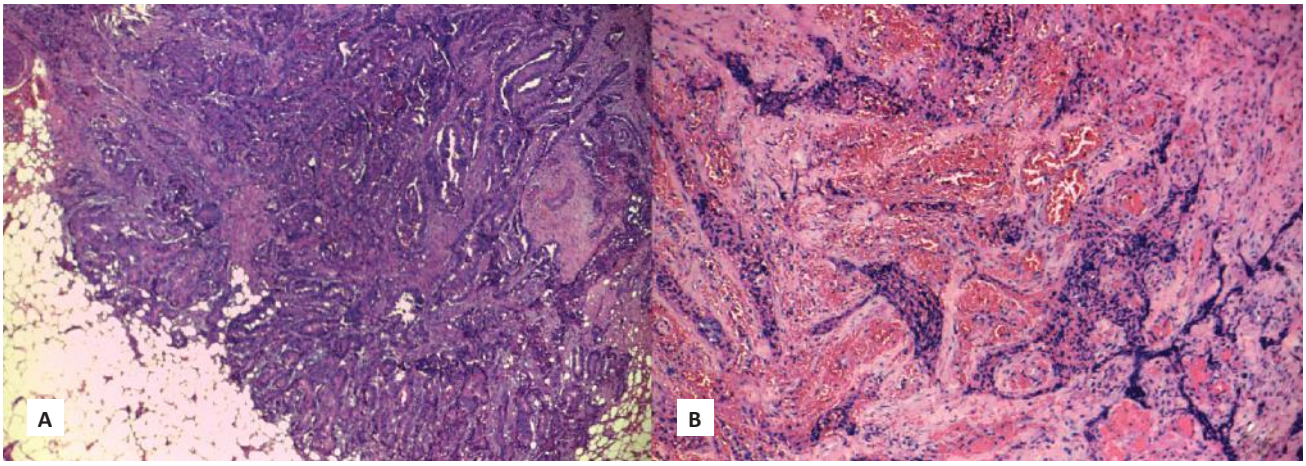


Figure 2. Histopathologic features of the high-density mass seen in the right breast of the patient discloses invasive ductal carcinoma. **(A)** Seen are neoplastic ductal structures infiltrating the surrounding structures including the adjacent adipose tissue (H&E, 40x). **(B)** Other areas prominently show the formation of new blood vessels amidst clusters of tumor cells with associated extensive desmoplasia (H&E, 100x).

or known allergy to iodine contrast media. The creatinine level is obtained at least a week before the scheduled exam. Ideally, for premenopausal women, the timing of the exam should coincide with Days 7-14 of her menses, to reduce background parenchymal enhancement.

An IV is inserted into the forearm or antecubital vein and a power injector at a rate of 3 ml/s infuses iodinated contrast agent. The volume is typically calculated at 1.5-ml/kg bodyweight. The iodine concentration ranges from 300 mg/ml to 370 mg/ml.

After infusion of the contrast media, let 2 minutes pass before positioning the patient for the standard mammographic views (Mediolateral Oblique and Craniocaudal views) of each breast. For each projection, two energy pairs – low energy and high energy are generated in a single compression. Severe acute reactions to contrast media occur in 4/10,000 (0.04%) patients.[14]

According to a study of Lalji et al., the low-energy CESM images are non-inferior to Full-Field Digital Mammography (FFDM) images with no significant differences in image quality, average glandular dose, and contrast detail.[15] The LE images therefore are equivalent to a standard mammogram. The additional radiation dose from the HE images in CESM was approximately 20% that of routine Full-Field Digital Mammography or the equivalent of 1 additional view.[10]

DISCUSSION

The core principle in functional imaging is based on tumor enhancement secondary to tumor neoangiogenesis. The process of tumor neoangiogenesis plays a central role in the growth and spread of tumors.[16] Tumor cells secrete vascular endothelial growth factor (VEGF), a potent angiogenesis activator that stimulates the formation and proliferation of endothelial cells.[17] The newly grown vessels are immature and differ from normal capillaries. They are tortuous and irregular, resulting in poorly efficient perfusion, they are leaky (especially to macromolecules), and they are independent of the normal mechanisms of regulation of the capillary blood flow. Hemodynamic characteristics of immature neovessels can be conservatively assessed by dynamic contrast-enhanced magnetic resonance imaging or computed tomography. Tissue enhancement depends on arterial input function, kinetics of distribution of blood into the capillary bed, leakage across the capillary walls, and volume of the interstitial space.[16]

In our current setting, the detection of breast cancer mainly utilizes analog film mammography, conventional digital mammography, digital breast Tomosynthesis (DBT/2D+3D Mammography) and breast ultrasound, which are based on morphologic (anatomic) information, as opposed to MRI (Magnetic Resonance Imaging) which gives functional information. MRI, however, is limited in its use in our local setting because of its limited availability and the cost is prohibitive.

The sensitivity of CESM was found to be high (98%), underscoring its potential to rival the diagnostic performance of MRI, but with added advantages of improved accessibility and lower cost.18 In women with dense breasts, Cheung et al., demonstrated that CESM is superior to mammography in both sensitivity and specificity with improvement from 71.5% to 92.7% and 51.8% to 67.9%, respectively.[19]

Akin to the improvements and technological advances made in the field of Pathology, namely, with the development of novel molecular characterization of breast cancer with cellular markers, functional-based imaging of breast cancer is poised to change the paradigm of current diagnostic practice.

Dual-energy Contrast-enhanced Spectral Mammography may provide added value in determining the microcalcifications that show enhancement with a diagnosis favorable to cancer or a lack thereof as virtually diagnostic for non-malignant or noninvasive subgroup of cancers.[20] Likewise, its ability to identify multifocal or multicentric disease enables it to adequately and simultaneously stage both breasts[21] for better pre-biopsy planning.

Local Experience

The first institute to acquire Contrast-enhanced Spectral Mammography is Health Cube Advanced Medical Imaging Unit in March 2016. It was initially utilized as part of the surveillance monitoring of patients with either mastectomy or post-breast conservation surgery and is currently integrated into the diagnostic workflow in the work-up of patients with clinically suspicious findings, particularly those who have dense breasts.

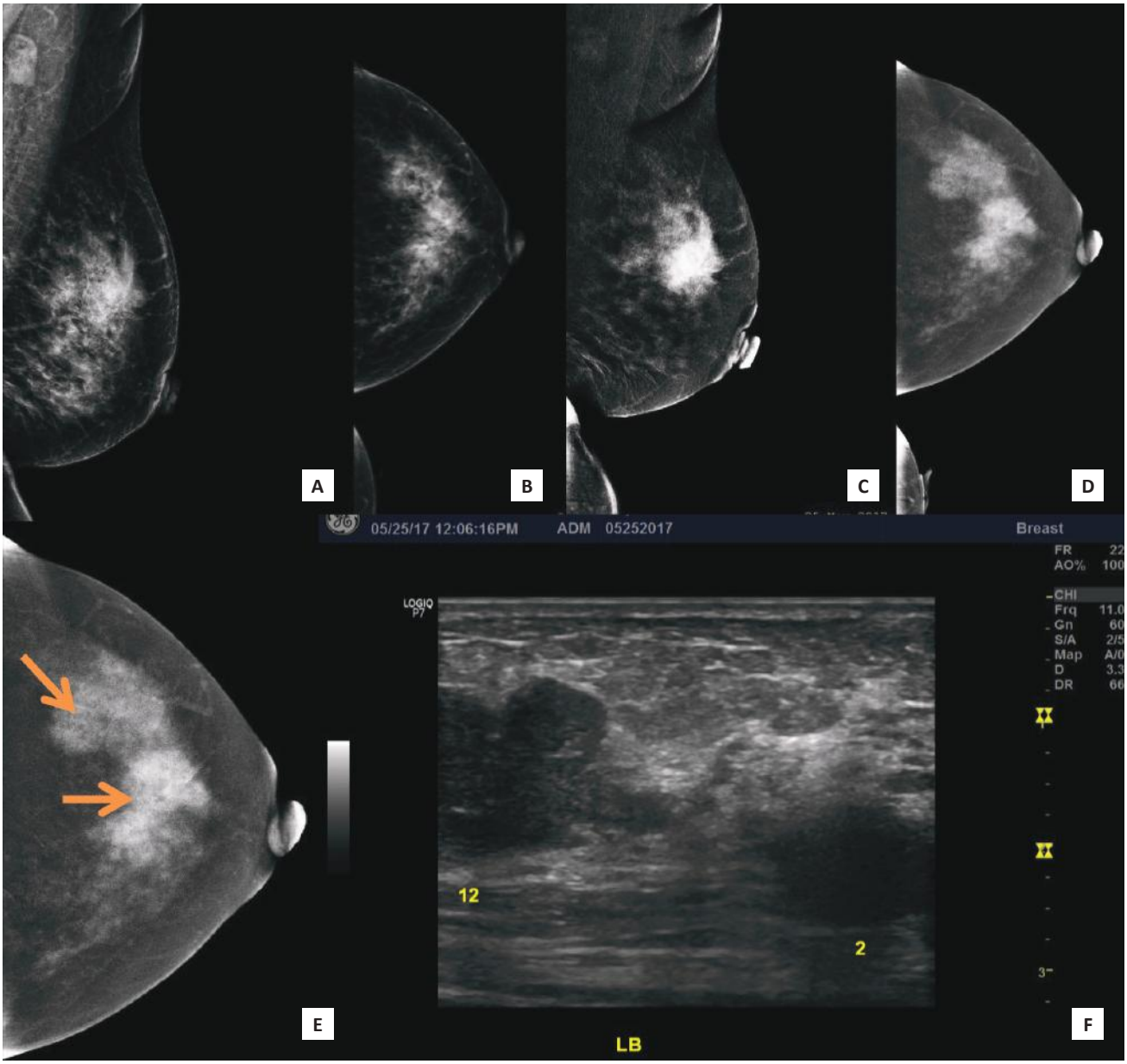


Figure 3. A 56-year-old premenopausal female presenting with a palpable left breast mass, Family history revealed she had two (2) sisters who had breast cancer in their 50's. **(A, B)** LE MLO and CC views of the left breast showing heterogeneously dense breast composition. There is an irregular mass of increased density at the mid upper quadrant and a second equal density mass with partly obscured margins at the upper outer quadrant **(C, D)** Subtracted images (SI) in MLO and CC projections show an enhancing irregular mass at the mid upper left breast and shows the second enhancing mass with lobulations at the upper outer quadrant on a background of moderate parenchymal enhancement. **(E, F)** SI Image of the left CC view and the correlate ultrasound image showing multifocal breast lesions at the 12 o'clock (orange arrow) and 2 o'clock (orange arrowhead) positions.

The two cases included in this case series showcase the ability of CESM to image cancer whether in dense or non-dense breasts. The first case illustrates cancer without background parenchymal enhancement in a non-dense breast while the second case shows two (2) foci of cancer in a patient with dense breasts with additional background parenchymal enhancement.

CONCLUSION

The trend towards molecular and functional-based diagnosis of breast pathology will continue and potentially become the standard of care in the near future, making it possible for a tailored and targeted approach in the improved management of breast cancer.

Contrast-enhanced spectral mammography stands to be a viable and practical diagnostic imaging modality that can contribute to increased cancer detection rate and improve breast cancer care in our country.

ACKNOWLEDGMENTS

The authors thank the following esteemed colleagues: Sherry L. Lee, MD, FPCS, FPSGS of Cardinal Santos Medical Center, and Timothy James Lam, MD of the University of the East Ramon Magsaysay Memorial Medical Center for the patient referrals and Paulo Giovanni L. Mendoza, MD, FPSP of Cardinal Santos Medical Center for his invaluable input on the histologic findings.

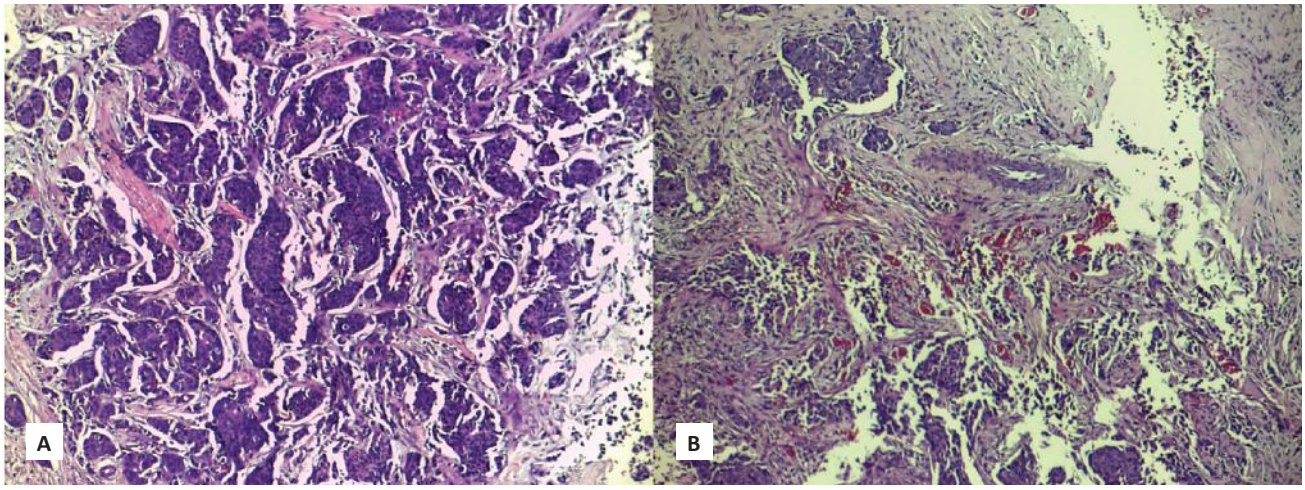


Figure 4. Microscopic appearances of the core biopsy taken from the enhancing left breast mass identified in the patient showing invasive ductal carcinoma. The tumor is very cellular and is composed of neoplastic cells disposed in tongues, cords and groups with attempts to form ducts **(A)** Note the pronounced desmoplastic reaction, which adds to the density seen radiographically. Some areas of the lesion reveal an abundance of well- and newly-formed blood vessels **(B)** (H&E, 100x).

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

FUNDING SOURCE

None.

REFERENCES

1. Jochelson M. Advanced imaging techniques for the detection of breast cancer. Am Soc Clin Oncol Educ Book. 2012:65-9. PMID: 24451711. https://doi.org/10.14694/EdBook_AM.2012.32.65.
2. Myers ER, Moorman P, Gierisch JM, et al. Benefits and harms of breast cancer screening: a systematic review. JAMA. 2015;314(15):1615-34. PMID: 26501537. https://doi.org/10. 1001/jama.2015.13183.
3. Carney PA, Miglioretti DL, Yankaskas BC, et al. Individual and combined effects of age, breast density, and hormone replacement therapy use on the accuracy of screening mammography. Ann Intern Med. 2003:138(3):168-75. PMID: 12558355.
4. Boyd NF, Guo H, Martin LJ, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med. 2007;356(3):227-36. PMID: 17229950. https://doi.org/10.1056/NEJMoa062790.
5. Yaghjyan L, Colditz GA, Collins LC, et al. Mammographic breast density and subsequent risk of breast cancer in postmenopausal women according to tumor characteristics. J Natl Cancer Inst. 2011;103(15):1179-89. PMID: 21795664. PMCID: PMC3149043. https://doi.org/10.1093/jnci/djr225.
6. del Carmen MG, Halpern EF, Kopans DB, et al. Mammographic breast density and race. AJR Am J Roentgenol. 2007;188(4):1147-50. PMID: 17377060. https://doi.org/10.2214/AJR.06.0619.
7. Lobbes MB, Lalji U, Houwers J, et al. Contrast-enhanced spectral mammography in patients referred from the breast cancer screening programme. Eur Radiol. 2014;24(7):1668-76. PMID: 24696228. https://doi.org/10.1007/s00330-014-3154-5.
8. Chang CH, Nesbit DE, Fisher DR, et al. Computed tomographic mammography using a conventional body scanner. AJR Am J Roentgeol. 1982;138(3):553-8. PMID: 6978009. https://doi.org/10.2214/ajr.138.3.553.
9. Lewin JM, Isaacs PK, Vance V, Larke FJ. Dual-energy contrast-enhanced digital subtraction mammography: feasibility. Radiology. 2003;229(1):261-8. PMID: 12888621. https://doi.org/10.1148/radiol.2291021276.
10. Jochelson MS, Dershaw DD, Sung JS, et al. Bilateral contrast-enhanced dual-energy digital mammography: feasibility and comparison with conventional digital mammography and MR imaging in women with known breast carcinoma. Radiology: 2013;266(3):743-51. PMID: 23220903. https://doi.org/10.1148/radiol.12121084.
11. Fallenberg EM, Dromain C, Diekmann F, et al. Constrast-enhanced spectral mammography vs. MRI: initial results in the detection of breast cancer and assessment of tumor size. Eur Radiol. 2014;24(1):256-64. PMID: 24048724. https://doi.org/10.1007/s00330-013-3007-7.
12. Lee-Felker SA, Tekchandani L, Thomas M, et al. Newly diagnosed breast cancer: comparison of contrast-enhanced spectral mammography and breast MR imaging in the Evaluation of Extent of Disease. Radiology. 2017;285(2):389-400. PMID: 28654337. doi.org/10.1148/radiol.2017161592.
13. Patel BK, Gray RJ, Pockaj BA, Potential cost-savings of contrast enhanced digital mammography. AJR Am J Roentgenol. 2017;208(6):W231-7. PMID: 28379734. https://doi.org/10.2214/AJR.16.17239.
14. Katayama H, Yamaguchi K, Kozuka T, Takashima T, Seez P, Matsuura K. Adverse reactions to ionic and nonionic contrast media. A report from the Japanese Committee on

the Safety of Contrast Media. Radiology. 1990;175(3):621-8. PMID: 2343107. https://doi.org/10.1148/radiology.175.3.2343107.
15. Lalji UC, Jeukens CRLPN, Houben I, et al. Evaluation of low-energy contrast-enhanced spectral mammography images by comparing them to full-field digital mammography using EUREF image quality criteria. Eur Radiol. 2015:25(10):2813–20. PMCID: PMC4562003. https://doi.org/10.1007/s00330-015-3695-2.
16. Cuenod CA, Fournier L, Balvay D, Guinebretière JM. Tumor angiogenesis: pathophysiology and implications for contrast-enhanced MRI and CT assessment. Abdom Imaging. 2006;31(2):188-93. PMID: 16447089. https://doi.org/10.1007/s00261-005-0386-5.
17. Weis SM, Cheresh DA. Tumor angiogenesis: molecular pathways and therapeutic targets. Nat Med. 2011;17(11):1359-70. PMID: 22064426. https://doi.org/10.1038/nm.2537.
18. Tagliafico AS, Bignotti B, Rossi F, et al. Diagnostic performance of contrast-enhanced spectral mammography: systematic review and meta-analysis. Breast. 2016;28:13-9. PMID: 27161411. https://doi.org/10.1016/j.breast.2016.04.008.
19. Cheung YC, Lin YC, Wan YL, et al. Diagnostic performance of dual-energy contrast-enhanced subtracted mammography in dense breasts compared to mammography alone: interobserver blind- reading analysis. Eur Radiol. 2014;24(10):2394-403. PMID: 24928280. https://doi.org/10.1007/s00330-014-3271-1.
20. Cheung YC, Yuan YH, Lin YC, Lo YF, Tsai HP, Ueng SH, et al. Dual-energy contrast-enhanced spectral mammography: enhancement analysis on BI-RADS 4 non-mass microcalcifications in screened women. PLOS ONE. 2016;11(9):e0162740. https://doi.org/10.1371/journal.pone.0162740.
21. Bhimani C, Malta D, Roth RG, Liao L, Timney E, Brill K, Germaine P. Contrast-ehanced spectral mammography: technique, indications, and clinical applications. Acad Radiol 2017;24(1):84-8. PMID: 27773458. https://doi.org/10.1016/j.acra.2016.08.019.

Alocilja Magnetic Nanoparticles capture *Escherichia coli* O157:H7 isolates

Dodge Lim,[1,6,7] Rovi Gem Villame,[2] Gloria June Quiñones,[3] Dave de Vera,[3] Rebecca Notorio,[3] Lilia Fernando,[4,7] Evangelyn Alocilja[5,7]

[1]*National Tuberculosis Reference Laboratory, Research Institute for Tropical Medicine-Department of Health, Philippines*
[2]*Department of Food Science and Chemistry, College of Science and Mathematics, University of the Philippines Mindanao*
[3]*Electron Microscopy Laboratory, Research Institute for Tropical Medicine-Department of Health, Philippines*
[4]*Nano-Biotechnology Laboratory, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños*
[5]*Alocilja Nano-Biosensors Lab, Biosystems and Agricultural Engineering, Michigan State University*
[6]*RITM TB Study Group*
[7]*Global Alliance for Rapid Diagnostics (GARD)*

Key words: Alocilja magnetic nanoparticles, E. coli O157:H7, TEM images, cell capture

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 29 September 2017.
Accepted: 17 November 2017.
Published online first: 18 November 2017.
https://doi.org/10.21141/PJP.2017.018

Corresponding author: Dodge R. Lim, RN
E-mail: dodge.lim@gmail.com

INTRODUCTION

Escherichia coli O157:H7 (EcO157) is a notorious foodborne pathogen known to cause bloody diarrhea and can even lead to death. Current detection methods, though highly sensitive, are lengthy and labor-intensive thus an alternative that is simple, rapid, low-cost and equally sensitive is necessary. Hence, an enabling method is the use of functionalized Alocilja magnetic nanoparticles (AMN), known to have high surface reactivity and can easily capture target biomolecules without the use of antibodies, such as microbial cells, in crude samples by means of a magnet. AMN, patented after its inventor Dr. Evangelyn Alocilja, is composed of iron oxide/glycan core/shell structure with an average size of 180-450 nm and with superparamagnetic properties. AMN has been reported to capture *Salmonella enterica*, *Bacillus cereus* and *Mycobacterium smegmatis* without the use of antibodies or peptides.[1-4]

In this preliminary work, our group tested the ability of AMN to capture a model organism, *E. coli* O157:H7 isolates provided by the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños (UPLB) Accession No. 10308 from dairy cattle (*Bos taurus* L.) feces.[5] O157 latex agglutination test (Oxoid Ltd., Thermo-Fischer Scientific, UK) was used to confirm the identity of the isolates. This strain was characterized through polymerase chain reaction (PCR) to carry shiga toxin-producing genes such as *stx1* which causes severe and fatal disease.

To demonstrate this, 100 µL of AMN solution (5 mg/mL) was added to a tube containing 1 mL of a 5-hr old pure EcO157 broth culture (with an initial population of ~108 CFU/mL). The mixed solution was then serially diluted and spot plated on Tryptic Soy Agar (TSA) (18-24 hours, 37°C) to determine the cell population prior to AMN capture. A serially diluted tube was sealed and gently mixed by inversion followed by a 5-min incubation at ambient conditions to allow conjugation of AMN to the cells. Magnetic separation for 1 min was performed to immobilize the resultant AMN-EcO157 complex. The supernatant was aspirated and discarded, and the AMN-EcO157 complex was resuspended in phosphate buffered solution (pH = 7.4). Spot plating on TSA was done to determine the population of bound cells. The percent cell capture efficiency (%CCE) was calculated by dividing the $\log_{10}$ of CFU/mL of captured cells over the $\log_{10}$ of CFU/mL before capture. The average %CCE of AMN towards pure EcO157 is 88.1±1.5 at pH = 7.4.

In order to visualize the capture of EcO157 by AMNs, the residual samples were further analyzed through transmission electron microscopy (TEM). Samples (50 to 100 µL) were dropped onto

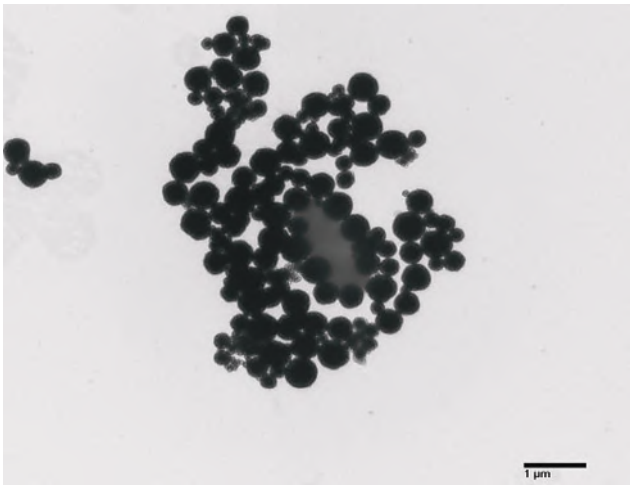


Figure 1. An AMN-EcO157 complex (TEM, mag: 2000x).

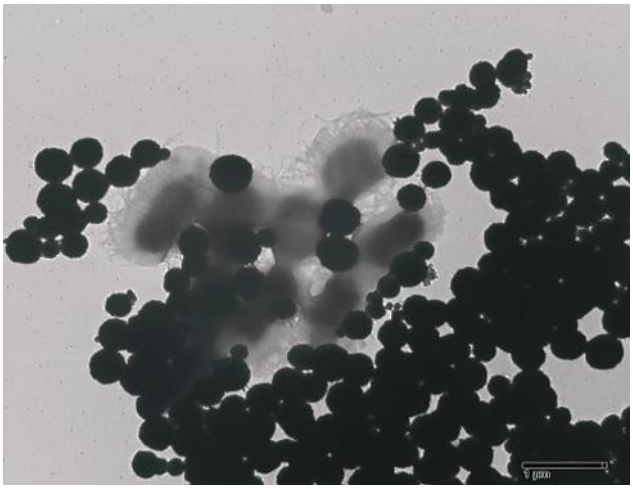


Figure 2. AMNs capturing aggregates of Ec0157 (TEM, 2500x).

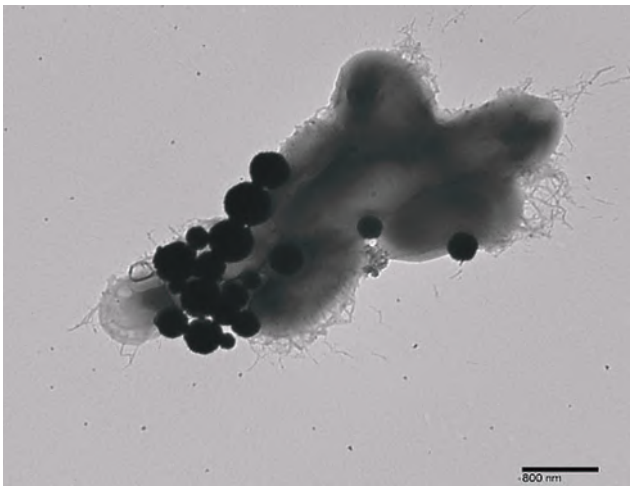


Figure 3. EcO157 captured by few AMNs (TEM, 3000x).

a 200 mesh formvar coated EM grids for 3 minutes at 25°C and drained. Grids were placed in a pre-labeled polystyrene petri dish lined with Whatmann filter paper and dried in electric desiccator cabinet overnight and examined using the JEOL JEM-1220 TEM under direct magnification of 2000x-3000x at 100 kV.

TEM micrographs confirmed conjugation between the AMPs and EcO157. Figure 1 shows that AMPs were effective in capturing the model pathogen by surrounding the entire cell. Aggregates of EcO157 were seen to interface with the AMNs in Figure 2. Also, it can be noted here that AMNs tend to heap up when far from biotic cells. In Figure 3, bacterial capture is evident despite the presence of a few AMNs.

Such capture of AMNs to EcO157 can be exploited to develop a potential for simple, centrifuge-free, preconcentration step for further downstream processing such as gDNA extraction, and sensitive DNA-based biosensor detection. Further studies are recommended to optimize test and apply AMN to capture cells from crude samples. The capture of AMNs to pathogens are governed by a number of biological phenomena, such as microbial adhesion, cell surface hydrophobicity, aggregation , biofilm formation, and surface to surface mediation such as hydrodynamic, Lifshitz-Van der Walls, electrostatic, acid-base and hydrophobic interaction forces.[6-8] AMN in solution increases particle density and surface area which promotes higher Brownian movement of the bacteria and AMNs.[6-8] Moreover, ionic and electrostatic interaction between the positively charged AMNs and negatively charged bacterial cell surface adds to the cohesive dynamics of the interaction.[9-10] Carbohydrate-binding proteins on bacterial cell wall also promotes aggregation and conjugation to nanoparticles.[11] Compounding all these interactive forces contributes to the synergy of cell capture by AMNs.

ACKNOWLEDGMENTS

We thank Dr. Socorro P. Lupisan, Dr. Amado O. Tandoc III, Dr. Ma. Cecilia G. Ama, Dr. Alpha Grace Cabic, Ms. Rocelle Marie Agero (Research Institute for Tropical Medicine); Dr. Rosario G. Monsalud, Dr. Francisco Elegado, David Joram Mendoza, Ma. Theresa Jonna A. Atienza, Ma. Theresa Perez (BIOTECH, UP Los Baños).

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

FUNDING SOURCE

This study was funded by the Research Institute for Tropical Medicine and is a recipient of the UP System *Emerging Interdisciplinary Research (EIDR) Grant*.

REFERENCES

1. Alocilja EC. Laboratory Research Division Brown Bag Presentation: nanotechnology for rapid diagnosis of infectious diseases. Metro Manila, Philippines: Research Institute for Tropical Medicine; 2017.
2. Zhang D, Carr DJ, Alocilja EC. Fluorescent bio-barcode DNA assay for the detection of Salmonella enterica serovar Enteritidis. Biosens Bioelectron 2009;24(5):1377–81. PMID: 18835708. https://doi.org/10.1016/j.bios.2008.07.081.
3. Vetrone SA, Huarng MC, Alocilja EC. Detection of Non-PCR Amplified S. enteritidis genomic DNA from food

matrices using a gold-nanoparticle DNA biosensor: a proof-of-concept study. sensors 2012;12(12):10487–99. PMCID: PMC3472839.
4. Pal S, Ying W, Alocilja EC, Downes FP. Sensitivity and specificity performance of a direct-charge transfer biosensor for detecting Bacillus cereus in selected food matrices. Biosyst Eng. 2008;99(4):461–8. https://doi.org/10.1016/j.biosystemseng.2007.11.015.
5. Rundina MCN. Isolation and characterization of Shiga (Vero) Toxin-Producing Escherichia coli O157:H7 in dairy cattle (Bos Taurus) from selected farms in Luzon, Philippines. College, Laguna, Philippines: University of the Philippines, Los Baños; 2004 [master's thesis].
6. Krasowska A, Sigler K. How microorganisms use hydrophobicity and what does this mean for human needs? Front Cell Infect Microbiol 2014;4:112. PMCID: PMC4137226. https://doi.org/10.3389/fcimb.2014.00112.
7. Xuan Y, Li Q, Hu W. Aggregation structure and thermal conductivity of nanofluids. AIChE J 2003;49(4):1038–43. https://doi.org/10.1002/aic.690490420.
8. Boks NP, Norde W, van der Mei HC, Busscher HJ. Forces involved in bacterial adhesion to hydrophilic and hydrophobic surfaces. Microbiology. 2008;154(10):3122–33. PMID: 18832318. https://doi.org/10.1099/mic.0.2008/018622-0.
9. van Loosdrecht MC, Lyklema J, Norde W, Zehnder AJB. Bacterial adhesion: a physicochemical approach. Microb Ecol. 1989;17(1):1–15. PMID: 24197119. https://doi.org/10.1007/BF02025589.
10. van Loosdrecht MC, Lyklema J, Norde W, Schraa G, Zehnder AJ. Electrophoretic mobility and hydrophobicity as a measured to predict the initial steps of bacterial adhesion. Appl Environ Microbiol. 1987;53(8):1898–901. PMCID: PMC204021.
11. dela Fuente JM, Penadés S. Glyconanoparticles: types, synthesis and applications in glycoscience, biomedicine and material science. Biochim Biophys Acta. 2006;1760(4):636–51. PMID: 16529864. https://doi.org/10.1016/j.bbagen.2005.12.001.

LABCON 2018

RESEARCH INSTITUTE FOR TROPICAL MEDICINE - DEPARTMENT OF HEALTH

8TH RITM LABORATORY POSTGRADUATE CONFERENCE
ON THE DIAGNOSIS OF INFECTIOUS DISEASES

BLAST

ELIMINATING INFECTIOUS AND TROPICAL DISEASES WITH DIAGNOSTIC, TECHNICAL AND SCIENTIFIC INNOVATIONS

15-16 March 2018 | The Bellevue Manila

REGISTER ONLINE
WITH CPD UNITS

EARLY BIRD RATE: **PHP 4,500**
(UNTIL 31 JANUARY 2018 ONLY)

REGULAR RATE: **PHP 5,000**

www.ritmlabcon.com
fb.com/ritmlabpostgrad
8thritmlabpostgrad@gmail.com
(02) 807-5890
09777742322 / 09995564858

CALL FOR ABSTRACTS

RITM LabCon 2018 invites abstract submissions for oral and poster presentations. Students, professionals and researchers are welcome to share their studies on infectious diseases.

** This advertisement is a complimentary service of the PJP for member societies/organizations.*

PHILIPPINE JOURNAL OF PATHOLOGY

Instructions to Authors

The **Philippine Journal of Pathology (PJP)** is an open-access, peer-reviewed, English language, medical and health science journal that is published continuously online and semi-annually in print by the Philippine Society of Pathologists, Inc. (PSP, Inc). All manuscripts must be submitted through the PJP Official Website (Open Journal Systems) (http://philippinejournalofpathology.org). All other correspondences and other editorial matters should be sent via electronic mail to philippinepathologyjournal@gmail.com.

Articles and any other material published in the PJP represent the work of the author(s) and do not reflect the opinions of the Editors or the Publisher. **Articles that do not subscribe to the Instructions to Authors shall be promptly returned.**

ARTICLE SECTIONS

The PJP welcomes manuscripts on all aspects of pathology and laboratory medicine, to include cytology, histopathology, autopsy, forensic pathology, clinical chemistry, clinical microscopy, medical microbiology, parasitology, immunology, hematology, blood banking, medical technology, laboratory diagnostics, laboratory biosafety and biosecurity, laboratory management, and quality assurance.

The PJP accepts original articles, review articles, case reports, feature articles, brief communications, autopsy cases, editorials, or letters to the Editor.

Original articles

The research must have received institutional review board approval that is explicitly stated in the methodology. The abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. A manuscript for original articles should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Reviews

Review articles, both solicited and unsolicited, provide information on the "state of the art." PJP reviews not only summarize current understanding of a particular topic but also critically appraise relevant literature and data sources, describe significant gaps in the research, and future directions. The abstract should be from 50 to 75 words and should not be structured. A manuscript for reviews should not exceed 15 typewritten pages (including tables, figures, illustrations and maximum of 50 references) or 4000 words.

Case Reports

This type of article pertains to single or multiple reports of well-characterized cases that are highly unusual, novel, or rare; or with a unique or variant presentation, evolution or course; or that represent an unexpected or uncommon association of two or more diseases or disorders that may represent a previously unsuspected causal relationship; or that are underreported in the literature. The abstract should be from 50 to 75 words and should not be structured. A manuscript for case reports should not exceed 10 typewritten pages (including tables, figures, illustrations and maximum of 15 references) or 3000 words.

Feature articles

The PJP may feature articles, either as part of an issue theme or a special topic on pathology by a local or international expert or authority. The abstract should be from 50 to 75 words and should not be structured. A manuscript for feature articles should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Autopsy Vault

The PJP highly welcomes articles on autopsy protocols of cases. The article must include a summary presentation of the history, evaluation and work-up, clinical course of a case, followed by the autopsy procedure performed, gross and microscopic findings, discussion, learning points and conclusion. The PJP recognizes the instructional and educational value of articles under this section. The abstract should be from 50 to 75 words and should not be structured. A manuscript for the Autopsy Vault should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Images in Pathology

Images of unique, interesting, or highly educational cases encountered in hematology, cytology, histopathology, or medical microbiology, may be submitted under this section, and may include photomicrographs, gross pictures, machine read-outs, among others. A brief history, the photograph(s) and short discussion of the case. No abstract is required. A manuscript for Images in Pathology should not exceed 500 words, with maximum of 10 references. This is distinct from the Case Report which is a full write up.

Brief Communications

Brief Communications are short reports intended to either extend or expound on previously published research or present new and significant findings which may have a major impact in current practice. If the former, authors must acknowledge and cite the research which they are building upon. The abstract should be from 50 to 75 words and should not be structured. A manuscript for brief communications should not exceed 5 typewritten pages (including tables, figures, illustrations and maximum of 10 references) or 1500 words.

Editorials

Recognized leaders in the field of pathology and laboratory medicine may be invited by the Editor-in-Chief/Editorial Board to present their scientific opinion and views of a particular topic within the context of an issue theme or issues on scholarly publication. No abstract or keywords necessary.

Letters to the Editor

PJP welcomes feedback and comments on previously published articles in the form of Letters to the Editor.

No abstract or keywords are necessary. A Letter to the Editor must not exceed 2 typewritten pages or 500 words.

Special Announcements

Special announcements may include upcoming conventions, seminars or conferences relevant to pathology. The Editors shall deliberate and decide on acceptance and publication of special announcements. Please coordinate with the Editorial Coordinator for any request for special announcements.

COVER LETTER

A cover letter must accompany each manuscript citing the complete title of the manuscript, the list of authors (complete names, position/designation and institutional affiliations), with one (1) author clearly designated as corresponding author, providing his/her complete institutional mailing address, institutional telephone/fax number, and work e-mail address. The **PJP Cover Letter Template** must be used.

PJP AUTHOR FORM

For submissions to the PJP to be accepted, all authors must read and sign the **PJP Author Form** consisting of: (1) the Authorship Certification, (2) the Author Declaration, (3) the Statement of Copyright Transfer, and (4) the Statement of Disclosure of Conflicts of Interest. The completely accomplished PJP Author Form shall be scanned and submitted along with the manuscript. No manuscript shall be received without the PJP Author Form.

GENERAL FORMATTING GUIDELINES

- Authors must use the standard PJP templates for each type of manuscript. These templates are aligned with the most current versions of the EQuaToR Network guidelines and checklists (http://equatornetwork.org).
- The manuscript should be encoded on the template using Microsoft Word (2007 version or later version), single-spaced, 2.54 cm margins throughout, on A4 size paper. Preferred fonts may include Century Gothic (template default), Times New Roman, or Arial.
- The manuscript should be arranged in sequence as follows: (1) Title Page, (2) Abstract, (3) Text, (4) References, (5) Tables, and (6) Figures & Illustrations.
- All the sheets of the manuscript should be labelled with the page number (in Hindu-Arabic Numerals) printed on the upper right corner.
- References should pertain directly to the work being reported. Within the text, references should be indicated using Hindu-Arabic numerals in superscripts.

SPECIFIC FORMATTING GUIDELINES

Title and Authors

- The title should be as concise as possible.
- A running title (less than 50 characters) shall also be required. The running title is the abbreviated version of the title that will be placed in the header. The running title should capture the essence of the manuscript title.
- The full name of the author(s) directly affiliated with the work should be included (First name, Middle initial and Last name). The order of authorship shall be the prerogative of the author(s).
- There are 4 criteria for authorship (ICMJE recommendations). These are captured in the **PJP Author Form**.
  - **Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND**
  - **Drafting the work or revising it critically for important intellectual content; AND**
  - **Final approval of the version to be published; AND**
  - **Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.**
- The highest educational attainment or title of the authors should be included as an attachment whenever appropriate (MD, PhD, et cetera).
- Name and location of no more than one (1) institutional affiliation per author may be included.
- If the paper has been presented in a scientific forum or convention, a note should be provided indicating the name of the forum or convention, location (country), and date of its presentation.

Abstract

- **For manuscripts under the "Original Article" section:** the abstract should contain no more than 300 words with a structured format consisting of the following standard headings: objective/s, methodology, results and conclusion.
- **For manuscripts under the "Feature Article," "Review Article," "Case Report," "Brief Communications," and "Autopsy Vault" sections:** the abstract should be no more than 200 words and need not be structured.
- Letters to the Editor and editorials do not require an abstract.

Keywords

At least three (3) keywords but no more than six (6), preferably using terms from the **Medical Subject Headings (MeSH) list of Index Medicus,** should be listed horizontally under the abstract for cross-indexing of the article.

Text

- The text should be organized consecutively as follows: **Introduction, Methodology, Results and Discussion, Conclusion** (IMRaD format), followed by **Disclosures, Acknowledgments** and **References.**
- All references, tables, figures and illustrations should be cited in the text, in numerical order.
- All abbreviations should be spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses. The same abbreviation may then be used subsequently instead of the full names.
- All measurements and weights should be in System International (SI) units.
- Under **Methodology**, information should be provided on institutional review board/ethics committee approval or informed consent taking (if appropriate).
- **Acknowledgements** to individuals/groups of persons, or institution/s who have contributed to the manuscript but *did not qualify as authors* based on the ICMJE criteria, should be included at the end of the text just before the references. Grants and subsidies from government or private institutions should also be acknowledged.

References

- References in the text should be identified by Hindu-Arabic Numerals in superscript on the same line as the preceding sentence.
- References should be numbered consecutively in the order by which they are mentioned in the text. They should not be alphabetized.
- All references should provide inclusive page numbers.
- Journal abbreviations should conform to those used in PubMed.
- A maximum of six authors per article can be cited; beyond that, name the first three and add "et al."
- The style/punctuation approved by PJP conforms to that recommended by the International Committee of Medical Journal Editors (ICMJE) available at http://www.icmje.org. Examples are shown below:

One to Six Authors

Krause RM. The origin of plagues: old and new. *Science*. 1992;257:1073-1078.

Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the US. JAMA. 2001;286(10):1195-1200.

More than Six Authors

Rhynes VK, McDonald JC, Gelder FB, et al. Soluble HLA class I in the serum of transplant recipients. Ann Surg. 1993; 217 (5): 485–9.

Authors Representing a Group

Moher D, Schulz KF, Altman D; for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA. 2001;285(15):1987-1991.

Book

Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

World Wide Web

Barry JM. The site of origin of the 1918 influenza pandemic and its public health implications. [Commentary]. JTranslational Med. January 20, 2004;2(3):1-4. http://www.translational-medicine.com/content/2/1/3. Accessed November 18, 2005.

Tables

- Cite all tables consecutively in the text and number them accordingly.
- Create tables preferably using Microsoft Excel with one table per worksheet.
- Tables should not be saved as image files.
- The content of tables should include a table number (Hindu-Arabic) and title in capital letters above the table.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the table. For legends, use small letters (i.e., a, b, c, d).
- Each table must be self-explanatory, being a supplement rather than a duplicate of information in the text.
- Up to a maximum of five (5) tables are allowed.

Figures and Graphs

- Figures or graphs should be identified by Hindu-Arabic Numeral/s with titles and explanations underneath.
- The numbers should correspond to the order in which the figures/graphs occur in the text.
- Figures & graphs should not be saved as image files. For illustrations and photographs, see next section.
- Provide a title and brief caption for each figure or graph. Caption should not be longer than 15-20 words.
- All identifying data of the subject/s or patient/s under study such as name or case numbers, should be removed.
- Up to a maximum of five (5) figures and graphs are allowed.

Illustrations and Photographs

- Where appropriate, all illustrations/photographic images should be at least 800 x 600 dpi and submitted as image files (preferably as .png, .jpeg or .gif files).
- For photomicrographs, the stain used (e.g. H & E) and magnification (e.g. X400) should be included in the description.
- Computer-generated illustrations which are not suited for reproduction should be professionally redrawn or printed on good quality laser printers. Photocopies are not acceptable.
- All letterings for illustration should be of adequate size to be readable even after size reduction.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the illustration/photograph.
- Up to a maximum of five (5) illustrations/ photographs are allowed.

N.B.: For tables, figures, graphs, illustrations and photographs that have been previously published in another journal or book, a note must be placed under the specific item stating that such has been adapted or lifted from the original publication. This should also be referenced in the **References** portion.

EDITORIAL PROCESS (Figure 1)

- The Editorial Coordinator shall review each submission to check if it has met aforementioned criteria and provide feedback to the author within 24 hours.
- Once complete submission is acknowledged, the manuscript undergoes Editorial Board Deliberation to decide whether it shall be considered or not for publication in the journal. Within five (5) working days, authors shall be notified through e-mail that their manuscript either (a) has been sent to referees for peer-review or (b) has been declined without review.
- The PJP implements a strict double blind peer review policy. For manuscripts that are reviewed, authors can expect a decision within ten (10) working days from editorial deliberation. There may be instances when decisions can take longer: in such cases, the Editorial Coordinator shall inform the authors.
- The editorial decision for manuscripts shall be one of the following: (a) acceptance without further revision, (b) acceptance with minor revisions, (c) major manuscript revision and resubmission, or (d) non-acceptance.
- Accepted manuscripts are subject to editorial modifications to bring them in conformity with the style of the journal. Copyediting and layout shall take five (5) working days, after which the manuscript is published online.
- All online articles from the last six (6) months shall be collated and published in print as a full issue.

EDITORIAL OFFICE CONTACT INFORMATION:

The Philippine Journal of Pathology
2nd Floor, Laboratory Research Division
Research Institute for Tropical Medicine
Filinvest Corporate City
Alabang, Muntinlupa City 1781
Editor-in-Chief: **Amado O. Tandoc III, MD, FPSP**
Telefax number: (+632)8097120
E-mail: philippinepathologyjournal@gmail.com
Website: http://philippinejournalofpathology.org

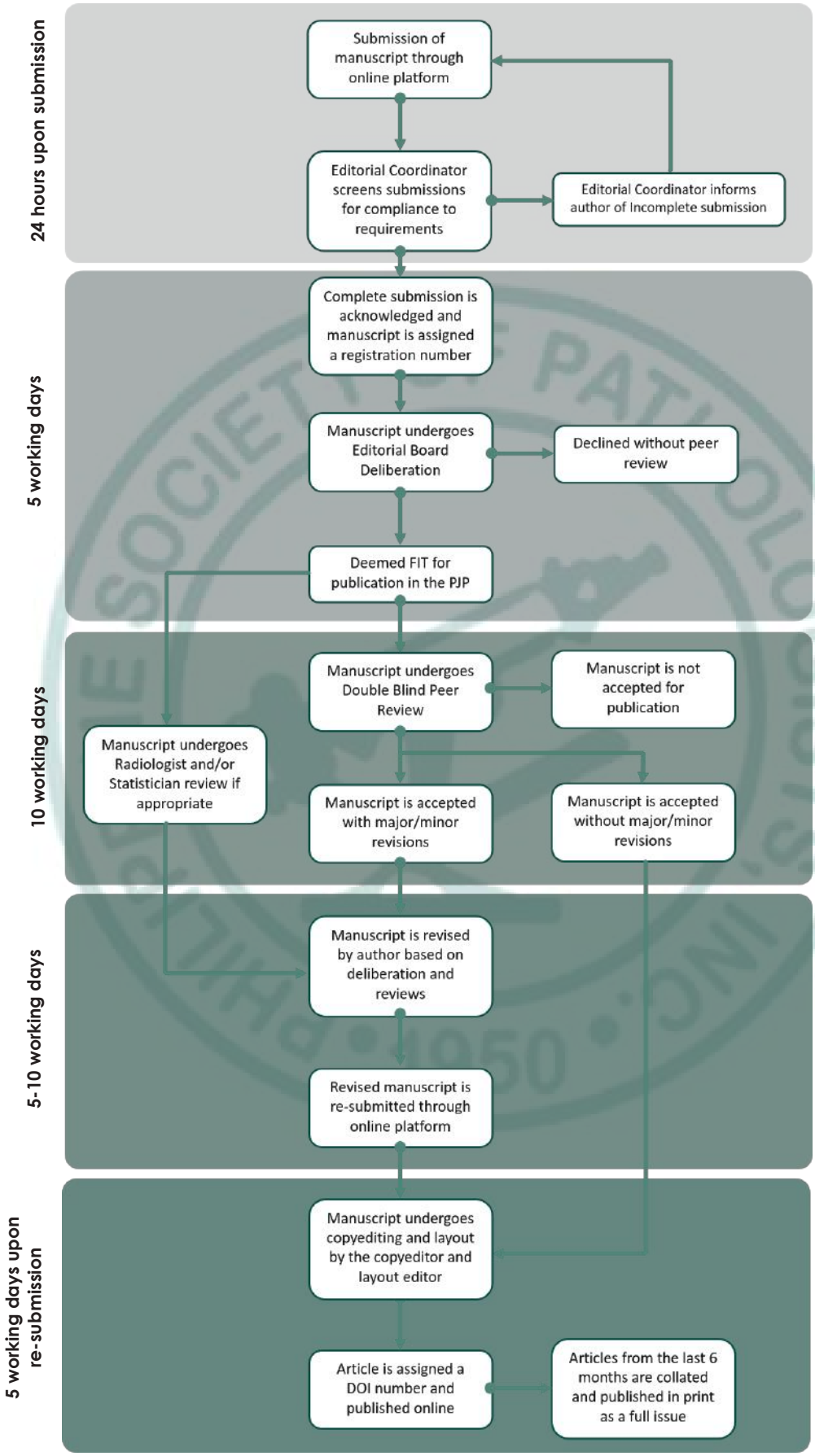


Figure 1. Editorial Process Flow.

PHILIPPINE JOURNAL OF PATHOLOGY
PJP

PJP AUTHOR FORM

For submissions to the PJP to be accepted, all authors must read and sign this PJP Author Form consisting of: (1) the Authorship Certification, (2) the Author Declaration, (3) the Statement of Copyright Transfer, and (4) the Statement of Disclosure of Conflicts of Interest. The completely accomplished PJP Author Form shall be scanned and submitted along with the manuscript. No manuscript shall be received without the PJP Author Form.

COMPLETE TITLE OF MANUSCRIPT

_____

_____

_____

AUTHORSHIP CERTIFICATION

☐ In consideration of our submission to the Philippine Journal of Pathology (PJP), the undersigned author(s) of the manuscript hereby certify, that all of us have actively and sufficiently participated in (1) the conception or design of the work, the acquisition, analysis and interpretation of data for the work; AND (2)drafting the work, revising it critically for important intellectual content; AND (3) that we are all responsible for the final approval of the version to be published; AND (4) we all agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

AUTHOR DECLARATIONS

☐ The undersigned author(s) of the manuscript hereby certify, that the submitted manuscript represents original, exclusive and unpublished material. It is not under simultaneous consideration for publication elsewhere. Furthermore, it will not be submitted for publication in another journal, until a decision is conveyed regarding its acceptability for publication in the PJP.

☐ The undersigned hereby certify, that the study on which the manuscript is based had conformed to ethical standards and/or had been reviewed by the appropriate ethics committee.

☐ The undersigned likewise hereby certify that the article had written/informed consent for publication from involved subjects (for case report/series only) and that in case the involved subject/s can no longer be contacted (i.e., retrospective studies, no contact information, et cetera), all means have been undertaken by the author(s) to obtain the consent.

AUTHOR STATEMENT OF COPYRIGHT TRANSFER

☐ Furthermore, the undersigned author(s) recognize that the PJP is an OPEN-ACCESS publication which licenses all published manuscripts to be used for building on and expanding knowledge, for non-commercial purposes, so long as the manuscripts are properly cited and recognized (Attribution-NonCommercial-ShareAlike 4.0 International Creative Commons License [CC BY-NC-SA 4.0]. The undersigned author(s) hereby, transfer/assign or otherwise convey all copyright ownership of the manuscript to the PJP.

AUTHOR DISCLOSURE OF CONFLICTS OF INTEREST

In order to ensure scientific objectivity and independence, the PJP requires all authors to make a full disclosure of areas of potential conflict of interest. Such disclosure will indicate whether the person and/or his/her immediate family has any financial relationship with pharmaceutical companies, medical equipment manufacturers, biomedical device manufacturers, or any companies with significant involvement in the field of health care. Place all disclosures in the table below. An extra form may be used if needed.

Examples of disclosures include but not limited to: ownership, employment, research support (including provision of equipment or materials), involvement as speaker, consultant, or any other financial relationship or arrangement with manufacturers, companies or suppliers. With respect to any relationships identified, author(s) must provide sufficiently detailed information to permit assessment of the significance of the potential conflict of interest (for example, the amount of money involved and/or the identification of any value of goods and services).

AUTHOR NAME	RELATIONSHIP	MANUFACTURER/ SUPPLIER/ COMPANY

All disclosures shall remain confidential during the review process and the nature of any final printed disclosure will be determined by the PJP. If there are no conflicts of interest to disclose, the author(s) should check the box below.

☐ I/We do not have any conflicts of interest to disclose.

Author Name	Signature	Date (MM/DD/YYYY)
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

PHILIPPINE JOURNAL OF PATHOLOGY
PJP

PATIENT CONSENT FORM

For case report and image submissions to the PJP to be accepted, the author/s must ensure that patients or patients' legal guardian/relative have provided informed consent to publish information about them in the journal. The completely accomplished PJP Patient Consent Form shall be scanned and submitted along with the manuscript. No case report and image shall be received without the PJP Consent Form.

Name of person described in article or shown in photograph:_____

_____

Subject matter of photograph or article (brief description):

_____

_____

_____

(The Subject matter of the photograph or article is hereafter termed as the "INFORMATION.")

Title of article:

_____

_____

I, _____ , give my consent for this information
[please insert your full name]

about MYSELF/MY CHILD OR WARD/MY RELATIVE relating to the subject matter
[please underline correct description]

above to appear in the Philippine Journal of Pathology (PJP) subject to its publication policies and ethical standards.

I have seen and read the material to be submitted to the PJP and thoroughly understand the following:

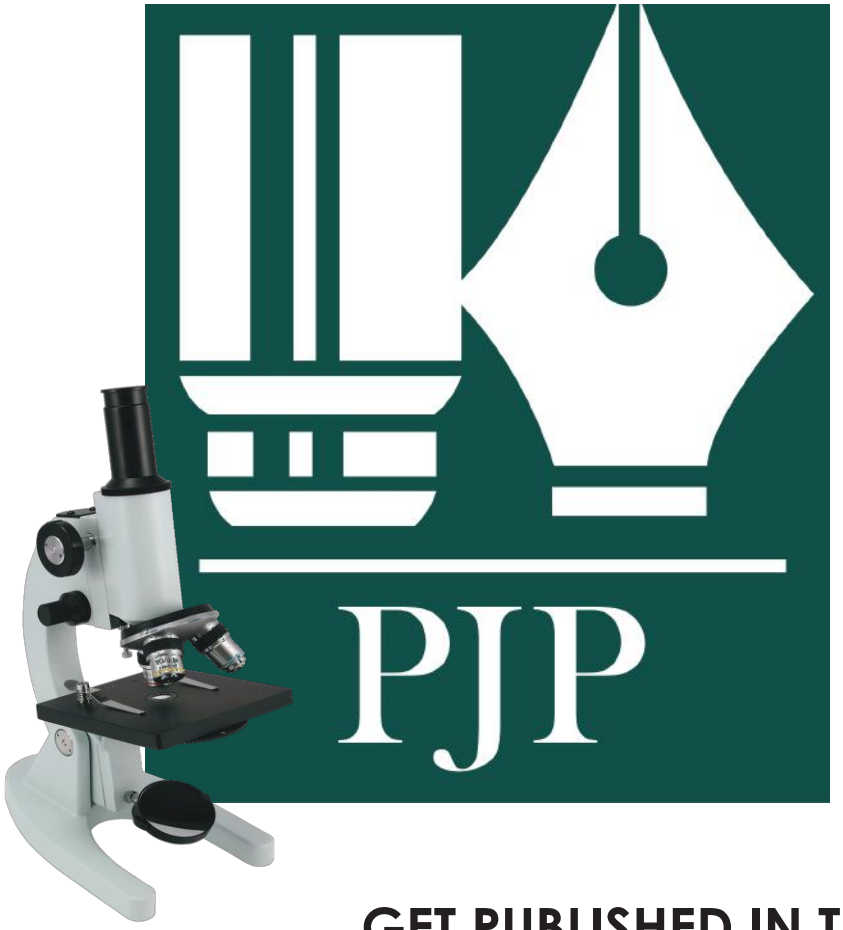
- The Information will be published in the PJP without my name. It is the obligation of the PJP to make all attempts, within its reasonable jurisdiction and authority, to ensure my anonymity.
- The Information may also be placed on the PJP website.
- The PJP shall not allow the Information to be used for advertising or packaging or to be used out of context (i.e., used to accompany an entirely different article or topic).
- I can withdraw my consent at any time before publication, but once the Information has already been sent to press, it is my understanding that it will not be possible to revoke the consent.

Signed:_____ Date:_____
[signature over complete name]

Witness:

Signed:_____ Date:_____
[signature over complete name]

PEER REVIEWERS



- *Online and Printed*
- *100% Open Access*
- *Peer Reviewed*
- *Continuous Publication model*
- *No Author Processing Fees*
- *Streamlined process from submission to publication*
- *24/7 web-based technical support*

GET PUBLISHED IN THE NEW PHILIPPINE JOURNAL OF PATHOLOGY!

The Philippine Journal of Pathology (PJP) is an open-access, peer-reviewed, English language, medical science journal published by the Philippine Society of Pathologists, Inc. It shall serve as the official platform for publication of high quality original articles, case reports or series, feature articles, and editorials covering topics on clinical and anatomic pathology, laboratory medicine and medical technology, diagnostics, laboratory biosafety and biosecurity, as well as laboratory quality assurance.

The journal's primary target audience are laboratorians, diagnosticians, laboratory managers, pathologists, medical technologists, and all other medical and scientific disciplines interfacing with the laboratory. For instructions and more information, visit our Official Website at:

http://philippinejournalofpathology.org

PJP Philippine Journal of Pathology

Philippine Society of Pathologists, Inc.

ICMJE International Committee of Medical Journal Editors

COPE Committee on Publication Ethics

equator network

Crossref

PAMJE

Philippine Journal of Pathology
Committee on Publications | Philippine Society of Pathologists, Inc.
E-mail: philippinejournalofpathology@gmail.com
Website: http://philippinejournalofpathology.org