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AD Express

NOVEMBER 2022

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From the Editors' Desk

Dear Reader,

It gives us immense joy to place the fourth edition of AD Express in your hands! Apollo Diagnostics (AD) has continued to shoulder arms along with the doyens of healthcare workers who have worked tirelessly to keep the COVID virus at bay & serve our patients in the best possible way.

The future augurs well for the pathology testing & we take this opportunity to thank all patrons for the immense support. While we gingerly await an imminent declaration by the WHO on the COVID pandemic, we thank the clinical as well as the laboratory fraternity for keeping the patient care ship afloat.

In the latest edition of AD express we have taken care to cover the commonplace as well as the 'cutting edge' aspects of laboratory medicine. We thank the contributors for taking time to put pen to paper & covering a gamut of topics with aplomb. Our editorial board also has decided by consensus to increase our frequency of publication & churn out issues on a monthly basis.

We are ever committed to our contribution to advanced diagnostics & we are pleased to inform that our association with SRMC, Chennai has progressed further. Whole-genome shotgun sequencing strain E. coli (CREC 2) has also been completed.

We humbly request you to share your feedback on 'AD express' & we assure you that feedback from you will make each next issue ever more interesting !

Best regards,

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Contents

Histopathology pitfall - Pseudocarcinomatous Hyperplasia of the Fallopian Tubes

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Introduction

Pseudocarcinomatous tubal hyperplasia or papillary tubal hyperplasia (PTH) by definition is the presence of small clusters of tubal epithelial cells and small papillae, with or without Psammoma bodies. These clusters and papillae are present within the tubal lumen and are usually associated with atypical proliferative serous tumour (APST) (1, 2).

Case presentation :

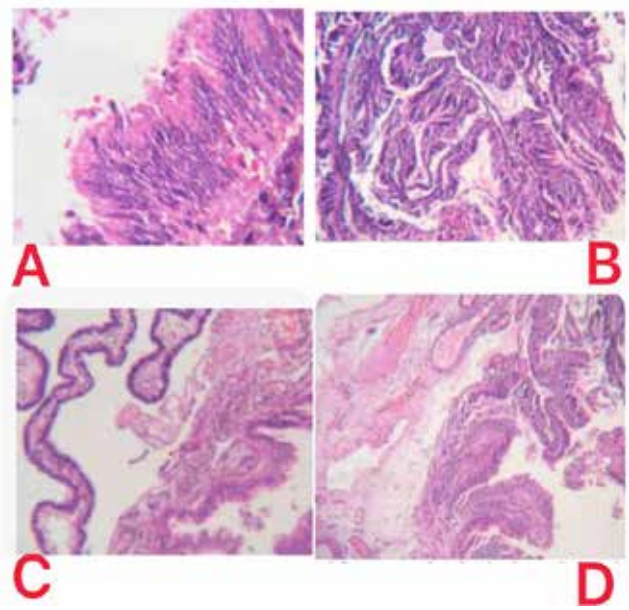
30 year old multipara

Pain abdomen & bleeding PV

H/O 2 Caesarean sections. One for PROM & second time for Pre-eclampsia culminating in abortion @ 5 weeks

Menstrual cycles were normal

Radiology : Ultrasound scan suggested a hyperechoic lesion & a possibility of ectopic pregnancy was suggested. With a clinical diagnosis of left tubo-ovarian mass, ectopic pregnancy, Laparoscopic left salpingo-oophorectomy with right fimbriectomy was done & specimen was sent for histopathological examination which revealed simple serous cyst of the ovary and pseudocarcinomatous tubal hyperplasia. No evidence of chorionic villi was found in the material studied.



A. High power view showing increased stratification of the lining ciliated epithelium.
B. High power view showing papillary hyperplastic epithelium with increased stratification of the lining epithelium
C: Low power view showing normal tubal ciliated epithelium on the left side and papillary hyperplastic epithelium on the right side.
D. Low power view showing hyperplasia and no invasion into the stroma

Discussion:

Tubal hyperplasias generally have no identifiable cause, but may be associated with inflammation, excess estrogen, ectopic pregnancy and neoplasia. The tubal epithelium may show nuclear crowding and stratification. There may be loss of nuclear polarity, tufting and varying degree of cytological atypia but cells maintain cilia and nuclear to cytoplasmic ratio. It is often an incidental finding, but its association with serous borderline tumors of the ovary has been suggested (3) .The process begins with chronic inflammation and leads to tubal hyperplasia, which then progresses to PTH .

Small papillae and clusters of cells from the fallopian tubes may implant on ovarian and peritoneal surfaces and may produce non-invasive epithelial implants, endosalpingiosis, atypical proliferative serous tumor (1). Robey & Silva identified tubal epithelial hyperplasia in about 70% of patients with ovarian serous borderline tumours (3).

Conclusion:

PTH is the most advanced stage of tubal hyperplasia and is associated with APSTs (1). The distal end of the fallopian tube could be exposed to inflammatory agents which results in likely repetitive damages and leads to precancerous lesions (4). Because of the role of the fallopian tube in ovarian carcinogenesis, few authors have suggested exclusive salpingectomy without associated oophorectomy (5). Thus, concurrent risk-reducing salpingectomies may become more widespread whenever a hysterectomy needs to be performed for benign indications (6). Further studies are still needed to better understand the various pre-neoplastic phases of ovarian cancer and the communication between the fallopian tube and the ovary (4). In our case ultrasound findings were not consistent with histopathological findings.

Histopathological examination plays an important role in reaching a definite diagnosis. Patient needs to have close follow up to identify any early changes towards ovarian malignancy and for a better prognosis.

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The gardener's copper pennies – Chromoblastomycosis presenting as a cystic lesion

Dr. Marquess Raj (ZTC - TN),
Dr. Chidhambharam.C (Consultant histopathologist, Chennai)

Introduction:

Chromoblastomycosis is caused by saprophytic, pigmented fungi commonly isolated from plant debris & soil. Clinical presentation of chromoblastomycosis is usually as a verrucous plaque or nodule (1). There are plenty of case reports & case series from South America on Chromoblastomycosis. In most case series, there has been a predilection for the lower limbs. However involvement of the upper limbs is not uncommon. The face, breast & tonsils are rarely infected (1).

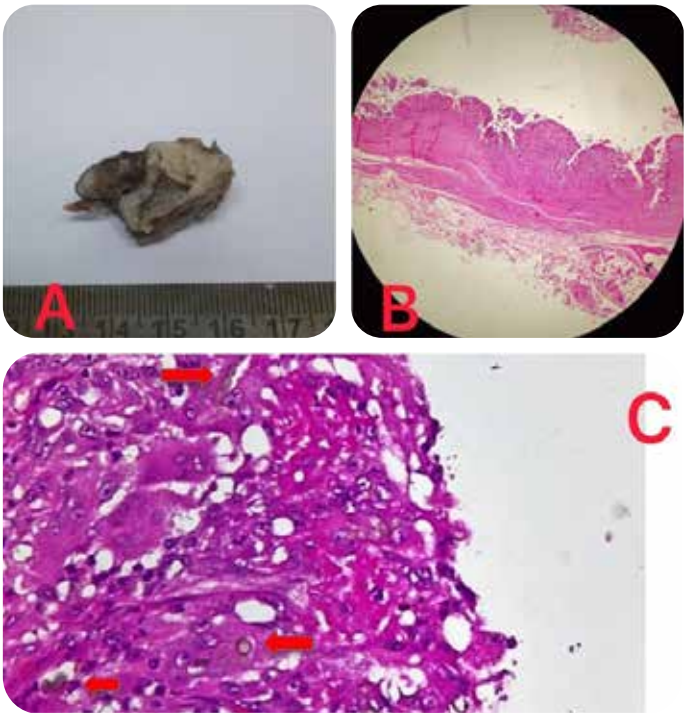
Irrespective of the site of involvement the presentation of chromoblastomycosis is usually as a verrucous lesion. We share a case of chromoblastomycosis presenting as a cystic lesion.

Case description:

A 45 year old male presented with a swelling in the left forearm. He was a gardener by occupation & apparently noticed a swelling in his forearm since 2 weeks. He was a diagnosed with diabetes mellitus since 2 years and is on regular medication for the same. The swelling was excised by the surgeon & a clinical diagnosis of infected bursa was given.

Histopathology:

The appearance of chromoblastomycosis is striking on histopathology is very similar to sporotrichosis. Hyperkeratosis, pseudo-epitheliomatous hyperplasia & granulomas in the upper & mid dermis are described (2). Tuberculoid granulomas with chronic inflammation as well as micro abscesses are described in chromoblastomycosis. Round, thick walled, golden brown cells described as 'sclerotic bodies' or 'medlar bodies' or 'muriform bodies are distinctly noted'. These bodies were noted in routine histopathology.



A. A cystic lesion was evident on cutting.
B. Cyst in low power. Blue arrows - inner surface with revealed plenty of macrophages on probing in high power.
C. Red arrows - Sclerotic bodies which appear singly or as stacks of round, brown coloured cells are noted. Many giant cells are also noted.

Discussion:

Chromoblastomycosis is a chronic deep cutaneous fungal infection usually affecting the site of inoculation. Presentation of skin lesions can take a long time, even decades (2). The usual presentation is a verrucous growth. Chromoblastomycosis presenting as a cystic lesion is unusual & is seldom described in literature. The clinician also suspected bursitis in this case & the gross specimen also revealed a cyst. A correlation with HLA-A29 suggests genetic factors may play a role, as well (2).

Occasional case reports describe squamous cell carcinoma arising in long standing lesions.

Causative agents include several fungi found in soil, wood and decaying plant material, hence, gardeners are susceptible –

- Fonsecaea pedrosi (most common pathogen, accounts for > 90% of the cases)
- Fonsecaea compacta
- Cladosporium carrionii
- Rhinocladiella aquaspersa (Ramichloridium cerophilum)

Unlike other fungi, special stains such as GMS & PAS are seldom described in the demonstration of chromoblastomycosis. Scraping of the lesion & demonstration of the fungus in a KOH preparation yields good results.

Treatment is difficult & long. Therapy can fail in upto 10 % of cases.

References:

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Folic deficiency - Does it really affect morphology of PAP smear

Dr. Aditi Parekh
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Foreword :

Folic acid plays an important role in synthesis as well as protection of our genetic code. Both methyl-THF and methylene-THF play critical roles in methylation — the donation of methyl groups in the synthesis of amino acids, and DNA and RNA nucleoproteins. These methyl groups attach to sections of the genetic code, preventing DNA damage and chromosome breakage, protecting our genome integrity by disallowing the expression of inappropriate developmental and disease causing genes (1).

Because folic acid functions at the cellular level to help prevent DNA damage and chromosomal breakage, it is not surprising that deficiency of folic acid paves way to a wide range of diseases and developmental disorders.

Scientific excerpts

Folic acid also works indirectly with t-RNA, helping to transport specific amino acids to their appropriate locations in the chain of forming protein molecules (2). Thymine is a part of DNA's structure and folic acid enzymes are needed to make thymine. When thymine is diminished, chromosome breakage occurs when DNA is stressed by viruses & carcinogens. Folic acid deficiency may be a precursor for transmission stage of HPV in cervical cells, and enhance its progression. Several studies indicate HPV is more easily contracted when folate levels are low (3).

Folate deficient cells are weak, and unable to achieve the same integrity, stability, and their full programmed of size and shape as coded by their parent genes. The morphology of these cells existing in this suspended states can manifest as asynchronous N: C maturation & even as cells with dysplastic features.

Folate gene mutations are a common reason for deficiency states, having been found in virtually every population tested world-wide thus far. It is also worth noting that folates in food are easily and often completely destroyed through oxidative damage during cooking and processing (3). Furthermore, our bodies do not produce folic acid, or store it for very long (>3 days), and adequate dietary intake is difficult to obtain, even with vigorous consumption of folate-rich food.

Certain polymorphisms in the folic acid converting enzyme, methylene-THF reductase (MTHFR), appear to be responsible for many deficiency states, and for enhancement of carcinogenesis, especially in the colon (4).

Studies have linked some cervical and endometrial neoplasms to folate gene mutations as well. High homocysteine, low folate, and DNA hypomethylation have been correlated with the progression and severity of cervical lesions from primordial stages (LSIL) to full blown cancer (5).

Kwasniewska's studies on 552 women between 1993-1996 found that a folate deficiency acted as a promoter in the development of cervical lesions. In his studies, a deficiency coexisting with HPV increased the risk of developing cervical intraepithelial neoplasia (LSIL) by 7 times!

The common inherited folate gene mutation, MTHFR 677 C-->T, has recently been identified in cervical and endometrial neoplasms. In experiments with a certain cervical cancer cell line (SW756), researchers identified a specific site on a certain chromosome (chromosome 12 at band q13) where HPV often inserts itself. This site is also the same site made fragile when folate enzymes are lacking.

Folic acid therapy has been effective in reversing some mild atypias, especially in women on hormone contraceptives. High levels of folic acid appear to provide a protective effect against the initiation of HPV related dysplasias (5).

Take home message:

- Improving folate levels in women at risk of getting infected or who are already infected with high-risk HPV may have a positive impact in the prevention of cervical cancer.
- High folate blood levels have been shown to provide protection against abnormal cytology smears in women taking oral contraceptives & have a protective effect in the development of carcinogenesis.
- Folic acid estimation along with serum homocysteine & vitamin B12 levels not only gives an indication of the nutritional status of the individual but also the propensity to develop potential cancers.
- It is recommended that women should consume not only folate-rich foods such as fruits and vegetables but also vitamin B12-rich foods such as meat, fish, milk products and eggs in a balanced way.

References:

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2. Mechanism and Regulation of Protein Synthesis in Saccharomyces cerevisiae
3. The Art of Cytology: An Illustrative Study Guide with Micronutrient discussions4. Influence of folate status on genomic DNA methylation in colonic mucosa of subjects without colorectal adenoma or cancer.
5. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives

The sunny side of Vitamin D

Dr. Marquess Raj (ZTC – TN & Pondicherry),
Dr. Srivatsan. R M.D (Consultant biochemist, RRL, Chennai)

Foreword :

While there are conflicting studies on the utility of vitamin D supplementation, it continues to be prescribed as a go - to ambrosia in many disease states. The cardio-protective, anti – cancer & even bone density enhancing properties of vitamin D are yet to be fully understood (1) However this does not entirely belittle the benefits of vitamin D supplementation. Cochrane datasets hold testimony to the advantage that vitamin D supplementation offers in pre-eclampsia, gestational diabetes & low birth weight babies. (2)

Nutritional rickets is a disease which has existed since medical history has been documented. Though vitamin D supplementation seems to thwart disease progression in children the same results are not evident when vitamin D supplements are given to adults.

The VITAL Bone Health study :

Many large RCTs have generated new results regarding the effects of vitamin D supplementation on the adult skeleton. The VITAL Bone Health study, aimed at evaluating the effects of vitamin D on bone structure and architecture, is a well-known example among physicians. The study included a cohort of 771 participants (men aged over 50 years and women aged over 55 years who had never taken vitamin D supplements, in their lifetime. The same subjects were given supplements & evaluated at baseline and after 2 years. Supplemental vitamin D (compared with placebo) had no effect on 2-year changes in real bone mineral density (BMD) at the spine, femoral neck, total hip or whole body, or on measures of bone structure. This conclusion remained valid in a subgroup analysis, including individuals with the lowest vitamin D status as measured by total at baseline.

New technology allows the direct measurement of free (non-protein-bound) as an alternative strategy to define vitamin D status. In participants of the VITAL trial with the lowest directly measured free concentrations, vitamin D supplementation generated a slight increase in spine are al BMD (0.75% in the vitamin D group versus 0% in the placebo group; P = 0.043) and attenuation in loss of total hip areal BMD (-0.42% in the vitamin D group versus -0.98% in the placebo group; P = 0.044). Clinical significance & implication of this marginal increase is debatable. (3)

Take home message :

- Vitamin D is a nutrient which plays a pivotal role in homeostasis beyond the musculoskeletal system.
- Genetic mechanisms & genes responsible for Vitamin D synthesis are being extensively studied & estimation of 25 levels is becoming ever increasingly common.
- It could be hypothesized that genetic mechanisms can determine the bio-availability of vitamin D.
- A single yardstick such as estimation of levels cannot be applied to determine candidates for vitamin D supplementation.
- There could be underlying genetic mechanisms behind certain individuals not showing increase in vitamin D levels even after taking supplements.
- With advanced tools such as next generation sequencing & microarrays genetic, such genetic mechanisms are likely to be studied with more rigor & our understanding of the sunshine vitamin is bound to grow in the near future.

References:

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3. Bikle, D., Bouillon, R., Thadhani, R. &Schoenmakers, I. Vitamin D metabolites in captivity? Should we measure free or total 25(OH)D to assess vitamin D status? J. Steroid Biochem. Mol. Biol. 173, 105–116 (2017).

Article - Quality Corner

Need for robust pre-analytical quality control to ensure maximum sensitivity of detection and clinical relevance in blood culture testing

Dr. Abhik Banerjee
Zonal Technical Chief, East Zone

Blood culture is probably the most common and one of the most critical specimens handled by the microbiology department in a medical laboratory. Blood culture being the most sensitive method for detecting blood stream infections (bacteremia, sepsis etc.) often guides antimicrobial therapy and thereby impacts patients' management to a great extent.

There are several pre-analytical variables that may significantly affect blood culture results. Hence, it is very important to design a robust quality control program including pre-analytical area to ensure best quality results in this segment.

As per various literature and current guidelines, pre-analytical factors like time of ordering the test in context to patient's clinical status, sample collection process, number of blood culture sets utilized, blood to broth ratio and many others may significantly influence the sensitivity of this test which must be recognized by the laboratory performing blood culture. Appropriate corrective and preventive actions should be taken by laboratory in case of any deviation to ensure that, test results are no way compromised even for a single case.

Key takeaways :

Clinicians should be cognizant of the fact that, sample contamination directly impacts blood culture report quality and thereby delays diagnosis in patients of suspected sepsis. Adequate and correct way of **disinfection of skin** at puncture site is essential. Hospital administrators must conduct regular and periodic training sessions (including live demonstrations, short videos) for phlebotomists and nursing staffs to ensure that, correct protocols like skin disinfection and **order of blood draw** are being followed at all times to mitigate the risk of contamination. Retraining and atleast annual competency assessment should be conducted and recorded to monitor the efficacy of training programs.

Both laboratory professionals and physicians should be aware of pre-analytical variables which affect blood culture results. An incorrect (both false negative and false positive) culture report may prove detrimental to patients' overall therapeutic outcome and henceforth, must be avoided at any cost. This is a collective responsibility of laboratory as well as treating physician.

Applications of Droplet digital PCR for screening in disease prognosis

Dr. Chittaranjan Jena
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Preface:

The Droplet digital polymer chain reaction (ddPCR) is a novel technology, newly commercialised to enable the accurate quantification of target nucleic acids in a sample. Digital PCR is preferable for identification of minor amounts of DNA targets such as point mutations, chromosomal translocations, DNA methylation and alternatively spliced mRNA. This technology also has a role in areas such as oncology diagnostics, non-invasive prenatal diagnostics, quantification of viral load, and assessment of microbial resistance.

Principle of ddPCR Technology:

Droplet digital PCR technology (ddPCR) technology uses as a combination of microfluids and proprietary surfactant chemistries to divide PCR samples into water-in-oil droplets. Droplets are formed in a water-oil emulsion to from the partition that separate the template DNA molecules. Individual droplets essentially function as individual test wells in a plate in which the PCR reaction takes place. The ddPCR system partitions nucleic acid samples into thousands of nanolitre sized droplets (20,000 droplets) and PCR amplification occurs within each droplet

The droplets support PCR amplification of the template molecules and use reagents with workflow similar to those used for most standard TaqMan probe-based assays. Following PCR, each droplet is analysed or read to determine the fraction of PCR positive droplets in the original sample. All the generated data are analysed by using Poisson statistics to determine the target DNA template concentration in the original samples.

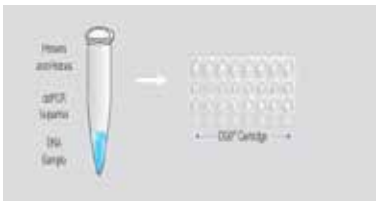
Workflow of PCR ready Samples:

Extraction of nucleic acids

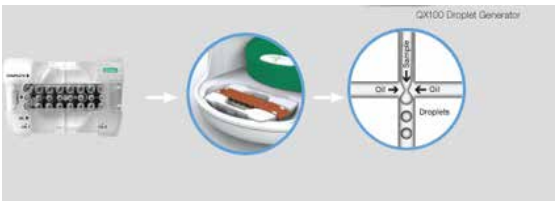
Droplet generation

PCR amplification of droplets

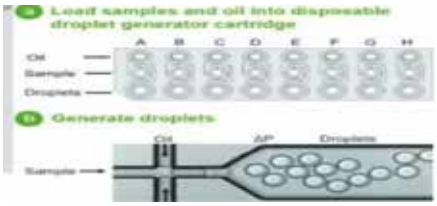
Droplet reading & analysis



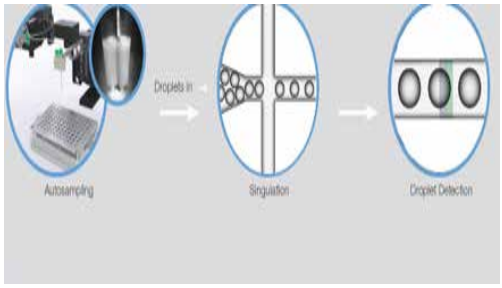
a. Prepare PCR ready sample



b. Droplet generation



c. PCR amplification of droplets



d. droplet reading

e. Analysis

Applications of ddPCR:

The Sample separation partitioning allows the sensitive, precise detection of a single template molecules and accurate quantification. DDPCR also eliminates the effect of target competition, construction of PCR amplification less sensitive to inhibition. Digital PCR propositions the benefits of absolute quantification and significantly enhanced sensitivity. Therefore, its application in the following areas is now increasing:

1. **Absolute quantification**
2. **Genomic alternations/copy number variation (CNV's)**
3. **Rare mutation/sequences detection (RMD)**
4. **Gene expression and microRNA analysis**
5. **Next Generation sequencing (NGS)**
6. **Single cell analysis**
7. **Genome edit detection (CRISPR-Cas9)**

Advantages of ddPCR Technologies:

- Droplet Digital PCR surpasses the performance of earlier digital PCR techniques by resolving the previous lack of scalable and practical technologies for digital PCR implementation. Serial dilution is laborious and introduces the possibility of pipetting error; competing chip-based systems rely on complex fluidics schemes for partitioning.
- The ddPCR improves precision, sensitivity and reproducibility; work station for target DNA analysis workflow is simple and requiring ~ 1hr hands on time.
- Quantify the rare DNA targets in a large wild-type background and detect less than 2-fold difference of DNA targets between samples.
- ddPCR addresses these shortcomings by massively partitioning the sample in the fluid phase in one step. The creation of tens of thousands of droplets means that a single sample can generate tens of thousands of data points rather than a single result, bringing the power of statistical analysis inherent in digital PCR into practical application.
- Droplet Digital PCR System automates and the workflow of droplet generation, thermal cycling, droplet reading, and easy data analysis, data interpretation, making this technology accessible to the working laboratory.

1. Chromoblastomycosis is caused by:

a) Bacteria b) Fungi
c) Parasite d) Virus

2. Following are true about Folic acid deficiency except:

a) Folate gene mutations is one of the causes
b) Risk of HPV infection is more
c) Deficient cell shows asynchronous N:C maturation
d) Microcytic anaemia on Peripheral blood picture

3. Rickets is caused by deficiency of

a) Vitamin A b) Vitamin C
c) Vitamin D d) Vitamin K

4. Measures to be taken while taking Blood culture samples is/are:

a) Appropriate disinfection of venepuncture site
b) Order of draw to be followed
c) Correct culture bottles to be used
d) All of the above.

5. Following can be the applications of Droplet Digital PCR (ddPCR):

a) Quantification of nucleic acids
b) Gene Expression analysis
c) Genome edit detection
d) All of the above

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