PathFinders Club
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"Embrace the Prometheus path: Through the trails of Pathology, the flame of understanding persists, illuminating the hidden realms of healing"

In the ancient Greek myth of Prometheus, the Titan defied Zeus by stealing the sacred fire and bestowing it upon humanity. As retribution, Zeus condemned Prometheus to eternal suffering. Chained to a rock, his liver was devoured daily by an eagle, symbolizing the relentless cycle of agony. However, Prometheus, immortal, endured the torment, demonstrating the resilience required for the pursuit of knowledge and the defiance against oppressive forces. This enduring saga echoes the profound connection between sacrifice, enlightenment, and the indomitable spirit of human curiosity.

Contents:

(Daderot, 2021)

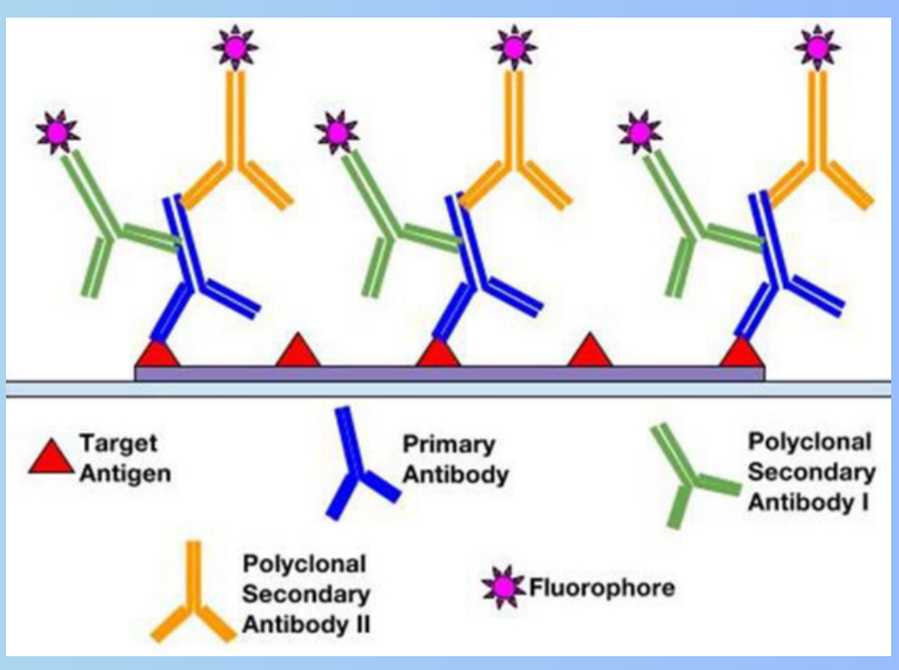

Figure 1. Amplification of signal with polyclonal secondary antibodies labeled with a fluorophore (Im *et al*, 2019)

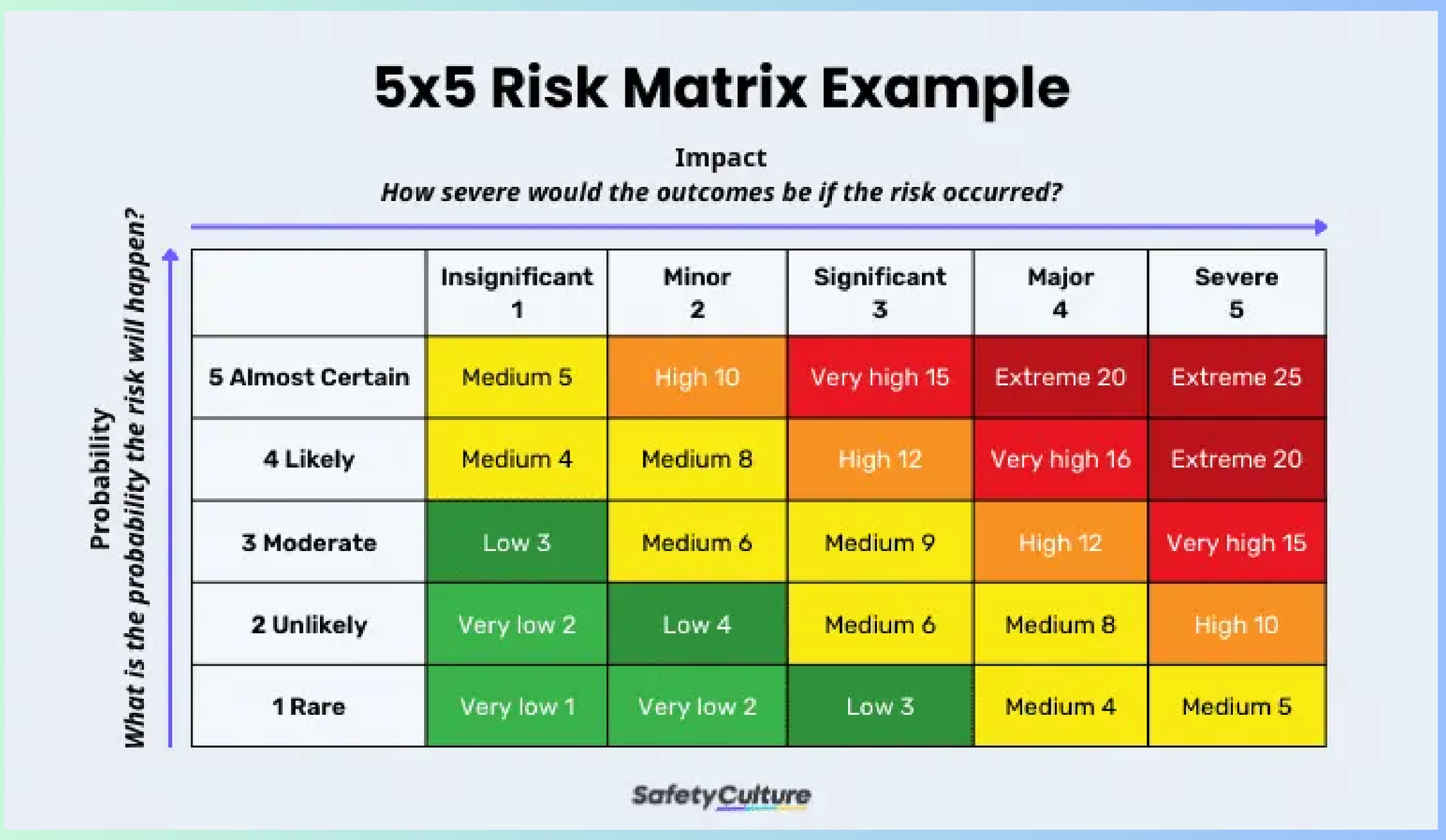

Figure 2. 5x5 Risk Matrix Example (SafetyCulture, 2023).

Introduction to RNAScope

-Ms. Vihanga Ranmini-

The developmental history of RNAScope represents a significant advancement in the field of RNA molecule detection. While other biomarker detection methods progressed over the years, RNA detection faced challenges due to RNA molecule instability. Techniques like Northern blotting, microarrays, qRT-PCR, qPCR, and traditional RNA in situ hybridization were developed but had limitations.In 2012, Advanced Cell Diagnostics introduced RNAScope, a commercially available in situ hybridization assay. RNAScope enables the detection of RNA in formalin-fixed paraffin-embedded tissues and tissue microarrays, allowing precise measurement of gene expression.This technique relies on RNA probes, specifically 'Z' probes, which consist of lower regions that hybridize to RNA, linker regions connecting to 'Z' probe tails, and 'Z' tails binding to pre-amplifier sequences. The 'Z' probe design ensures high specificity, single molecule detection, and reduced off-target binding, minimizing background noise.The workflow involves slide preparation tailored to the tissue type, followed by permeabilization, hybridization, and signal amplification. The results can be visualized using bright-field or fluorescent microscopy.RNAScope finds applications in neuroscience, cancer research, immuno-oncology, cell and gene therapy, and single-cell analysis. Its advantages include single-cell gene expression analysis, independence from antibodies, high sensitivity, specificity, and analytical accuracy. However, it is not suitable for discriminating viral RNA transcripts from viral DNA, and its cost is higher compared to immunohistochemistry (IHC).

Key References:

- De Biase D, Prisco F, Piegari G, Ilsami A, d'Aquino I, Baldassarre V, Zito Marino F, Franco R, Papparella S, Paciello O. RNAScope in situ hybridization as a novel technique for the assessment of c-KIT mRNA expression in canine mast cell tumor. Frontiers in Veterinary Science. 2021 Feb 16;8:591961.
- Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, Wu X, Vo HT, Ma XJ, Luo Y. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. The Journal of molecular diagnostics. 2012 Jan 1;14(1):22-9.

Comparison of RNAscope and Immunohistochemistry for Evaluation of the UPK2 status in Urothelialcarcinoma Tissues

-Dr. Isuri Yasara-

In the context of urothelial carcinomas (UCs), which constitute the majority of bladder cancers and often exhibit diverse histologic variants, the accurate determination of UPK2 status is crucial for diagnosis. Immunohistochemistry (IHC) has been a conventional method for this purpose but often falls short in terms of sensitivity. This study explored the potential of RNAscope, a novel technique, to enhance UPK2 detection in UC tissues.Uroplakin 2 (UPK2), an exclusive marker of normal urothelium, plays a vital role in distinguishing UC from nonurothelial tissues. The study compared UPK2 detection using IHC and RNAscope in tissue microarray sections. Surprisingly, the results revealed no significant difference in UPK2 detection between RNAscope (68.0%) and IHC (62.6%). A moderate positive correlation was observed between the two methods.This finding, contrary to some previous studies, suggests that for UPK2 detection, RNAscope does not significantly outperform IHC. Potential reasons for this discordance include the molecular characteristics of intratumor subtypes in UC, the imperfect correlation between mRNA and protein levels, and technical differences in protein and mRNA detection.While RNAscope offers promise, the study acknowledges limitations in patient selection and sample size. Future directions should involve whole-slide analysis to account for tumor heterogeneity and multicenter studies for broader validation. In conclusion, RNAscope demonstrates comparable performance to IHC for UPK2 detection, offering an alternative or adjunct method in clinical practice.

Key References:

- Lu J, Zhao M, Wu C, Chu C, Zhang CZ, Cao Y. Comparison of RNAscope and immunohistochemistry for evaluation of the UPK2 status in urothelial carcinoma tissues. Diagnostic Pathology. 2022 Dec;17(1):1-6.

Molecular Diagnostics of Colorectal Carcinoma

-Dr. Hiruni Seneviratne-

Colorectal carcinoma is a complex and heterogeneous disease. There are several oncogenic pathways involved in the carcinogenesis of colorectal carcinoma. Molecular diagnostics are helpful in detecting Lynch syndrome, predicting prognosis and making therapeutic decisions. Lynch syndrome is usually caused by germline mutations in mismatch repair (MMR) genes. Defects in mismatch repair genes result in microsatellite instability (MSI). The accumulation of DNA mismatches leads to an increased risk of developing malignant neoplasms. Universal screening for MMR deficiency is recommended for all newly diagnosed colorectal carcinomas. MSI testing is conducted with PCR or immunohistochemistry for MMR proteins. The EGFR signaling pathway plays an important role in the carcinogenesis of colorectal carcinomas due to the availability of EGFR-targeted therapy. KRAS gene mutational analysis is mandatory for candidates who are eligible for anti-EGFR therapy. Because the presence of KRAS gene mutations leads to poor response. All colorectal carcinomas should undergo comprehensive genetic analysis for somatic mutations and selected individuals are subjected to germline DNA testing. The rapid spread of next-generation sequencing technology is likely to affect current approach of colorectal carcinoma screening, diagnosis, treatment and monitoring in the future.

Key References:

- Shi C, Washington K. Molecular testing in colorectal cancer: diagnosis of Lynch syndrome and personalized cancer medicine. American journal of clinical pathology. 2012 Jun 1;137(6):847-59.
- Colon cancer biomarker testing (including MSI / Lynch) [Internet]. www.pathologyoutlines.com.Availablefrom:https://www.pathologyoutlines.com/topic/stainslynchcolorectal.html

Introduction to AI and its Applications

-Ms. W.G. Dinushi-

Computers can now simulate human intelligence via artificial intelligence (AI), which includes abilities like speech recognition, decision-making, and language translation. AI is split into narrow AI for specific tasks and general AI for versatile capabilities. Natural Language Processing is a standout, enhancing chatbots, language translation, and sentiment analysis. Breakthroughs like deep learning and models like GPT-3 are transforming AI. AI's transformative potential is vividly demonstrated in healthcare, where AI-powered algorithms excel in medical imaging, aiding in the identification of suspicious lesions and tumours. AI is reshaping the landscape of cancer detection through liquid biopsies, offering a non-invasive method for early cancer detection by detecting subtle genetic alterations associated with various cancer types. In organ transplantation, AI optimizes kidney matching, reducing wait times and improving success rates. It also aids in outcome prediction, reduces rejection risks, optimizes drug dosages, and enables telemedicine. AI is pivotal in neuroscience, advancing Brain-Computer Interfaces (BCIs) for direct brain-device communication, aiding those with neuromuscular disorders. AI also addresses brain tumours, neurodegenerative diseases, cerebrovascular accidents, and neurological infections in the field. However, AI also brings challenges, including biases in AI systems, privacy concerns, potential job displacement, transparency issues, security risks, and ethical dilemmas. Despite these challenges, AI's promise to enhance various aspects of our lives remains undeniable, promising a future where technology augments human potential and capabilities.

key References:

- Ardila, D., Kiraly, A.P., Bharadwaj, S., Choi, B., Reicher, J.J., Peng, L., Tse, D., Etemadi, M., Ye, W., Corrado, G. and Naidich, D.P., 2019. End-to-end lung cancer screening with three-dimensional deep learning on low-dose chest computed tomography. Nature medicine, 25(6), pp.954-961.
- Surianarayanan, C., Lawrence, J.J., Chelliah, P.R., Prakash, E. and Hewage, C., 2023. Convergence of Artificial Intelligence and Neuroscience towards the Diagnosis of Neurological Disorders—A Scoping Review. Sensors, 23(6), p.3062

Immunofluorescence Reporting in Histopathology

-Dr. Niluka Walgamage-

Immunofluorescence (IF) is a technique that permits visualization of virtually many components in any given tissue or cell type. This broad capability is achieved through combinations of specific antibodies tagged with fluorophores and allowing them to bind with target antigens. There are two methods available, depending on the specific antibodies in use; direct/primary and indirect or secondary. For IF evaluation a variety of sample conditions can be employed. Fresh samples followed by SNCP freezing is the most common sample condition that we used. Sample fixation, cell permeabilization, antigen retrieval, blocking of non- specific binding sites, primary antibody incubation, washing to remove unbound primary antibodies, secondarily antibody incubation, washing to remove secondary antibodies, counterstain mounting the slide are the basic steps in IF technique (Figure 1). In current practice, combining IF, haematoxylin and eosin (H and E) imaging requires to give a complete diagnosis. IF staining is mainly used to diagnose pathological conditions related to skin and kidney. Furthermore, IF is compulsory to make some of the pathological conditions such as IgA nephropathy and linear IgA dermatoses. Multiplex IF imaging and H and E staining of some tissue sections for the interpretation of the molecular data is the emerging topic in tumor pathology.

Key References:

- Im K, Mareninov S, Diaz MF, Yong WH. An introduction to performing immunofluorescence staining. Biobanking: methods and protocols. 2019:299-311.
- 2.Lin JR, Chen YA, Campton D, Cooper J, Coy S, Yapp C, Tefft JB, McCarty E, Ligon KL, Rodig SJ, Reese S. High-plex immunofluorescence imaging and traditional histology of the same tissue section for discovering image-based biomarkers. Nature Cancer. 2023 Jun 22:1-7.

Risk Based Quality improvement: Part 3

-Prof. K.A.C. Wickramaratne-

How to apply risks in to a matrix and how to give a quantification to the potential risk can be done by using different risk matrices. The laboratories can use any of these matrices as per their discretion. A matrix with 5X5 table is given in the front page (Figure 2). A table should be prepared with activities or processes, steps, how each step can go wrong, the likelihood of occurrence, consequences whether mild or serious. Based on that action has to be planned. After implementation of all the activities, residual risks as well has to be calculated. A table prepared for risk management is provided below as a link. In that, blood collection from drip arm in inward or ICU patients is taken as the activity which is wrong as we should avoid arm with IV drips from blood collection. But it happens. That causes serious errors, delays. So the laboratory has implemented some training, introduced checklists, procedural work charts, and developed policies on blood collection from drip arm etc. That gives more assurance, and confidence on reliability of laboratory test results and finally job satisfaction to laboratory team. Above all we can be happy thinking that our patients are safe! It is not complete from that point. It is mandatory to monitor and identify any potential risks if any!

(https://drive.google.com/file/d/1eRCSprr_jXosWOtefLz_DdAD3zZq9H_n/view?usp=sharing)

Key References:

1. ISO 15189:2022
2. EP 23-A A practical guide for laboratory Quality Control Based on Risk Management
3. EQUiP 7 ACHSi risk assessment and control