Liquid-Based Microbiology: the past, the present, the future

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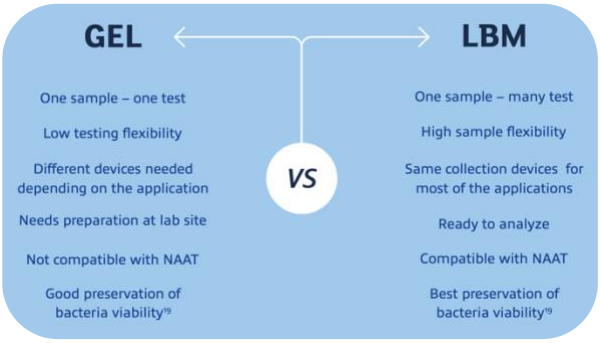
INTRODUCTION

In the last two decades, the world of microbiology underwent profound changes. Generally speaking, microbiology labs have seen an increased workload, and new pathogens have arisen; thus, new approaches needed to be developed and implemented, new diagnostics techniques have been developed, and a chronic lack of resources (both financial and human resources) forced labs to reshape and become more efficient and effective. All the phases of the diagnostics process were impacted in this process, including preanalytics.

Undoubtedly, one of the major improvements was the development of Liquid-Based Microbiology (LBM). This technology represented a departure from the conventional way to transport and preserve samples, from a solid format (a cotton or rayon swab inserted in a gelified transport media or a wet sponge) to a fully liquid solution, where the sample is released. Compared to the gel-based transport system, LBM introduces many benefits:



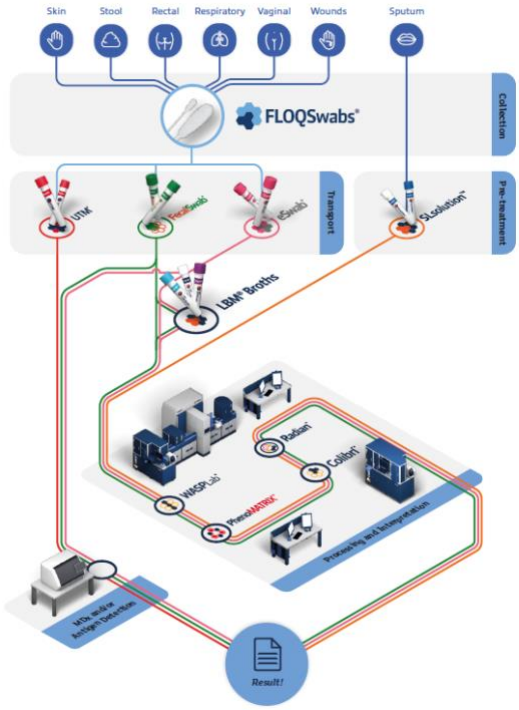
The uniform release of the sample into the transport media is one of the key features of LBM, and it is only possible by combining it with a FLOQSwabs®.

The benefits of moving from a gel-based transport system to LBM are not strictly related to the lab but can also be extended to the sample collection staff, the patients, and the procurement office, as noticeable in the table below:

All these improvements significantly impacted the microbiology diagnostics flow, making it easier, standardized, and automatable.

THE RIGHT PRODUCT FOR THE RIGHT APPLICATION

The modern microbiology lab is a mix of different technologies and platforms. Standardizing the sample collection using a few validated devices is possible and a real option. Said this, having the chance to choose different transport media characterized by different preservation specificities allows every professional to select the best transport media for any need.



This picture is meant only for flow demonstration purposes, and the data reported are not intended to replace the Instructions for Use (IFU). Always refer to the IFU for the final application compatibility or refer to a Copan representative. For molecular and antigen detection applications compatibility, refer to the device's manufacturer IFU.

CULTURE METHODS

The main objective for a bacteriology medium is to preserve the vitality of the microorganism that needs to be cultured, avoiding, at the same time, the overgrowth of commensal flora and high-fitness microorganisms. Intuitively, as the typology and physiology of the pathogens of human interest are very broad, designing a "universal media" is very complicated. Historically, at least three main gel-based transport systems were used according to the typology of bacteria to recover: Stuart or Amies gel w/o charcoal for aerobes, Stuart or Amies gel with charcoal for fastidious bacteria, and a specifically designed device for anaerobes. This high diversity of collection and transport devices definitively poses issues in terms of standardization and usability.

eSwab®

The Copan eSwab® was launched in 2006 and represented a fundamental improvement over the past transport system. In fact, with eSwab®, the microbiologist can standardize most of the needs on just one collection device. Copan eSwab® is based on liquid Amies and is designed to preserve the vitality of aerobes bacteria, fastidious bacteria, and anaerobes for up to 48 hours at room temperature (apart from *N. gonorrhea*, which is viable up to 24 hours). eSwab® was also proven to work efficiently for the viability preservation of fungi and yeast and for stabilizing DNA and antigens for molecular testing and antigen detection. eSwab® is the perfect combination with any automation platform for plate streaking and incubation.

eSwab® is currently used in routine by thousands of laboratories worldwide, and it was cited in over 180 peer-reviewed papers for multiple applications.

eSwab® was proven equivalent or superior to standard gel-based transport media in preserving bacteria viability and allowing Gram stain[1-2]. Results from stand-alone evaluations were excellent on aerobes on both mono species and poli species samples[3-4], on fastidious bacteria[5], and anaerobic bacteria[6].

Antibiotic Susceptibility Testing (AST) is now among the most important – if not the most important – microbiology tests run in the lab. eSwab® fits this purpose perfectly, as it allows the collection of samples from different body sites, fully preserving the viability of the researched bacteria for potential antibiotic phenotypic detection. eSwab® was extensively evaluated, and it is currently routinely used worldwide for collecting samples to research MRSA from wounds[7], skin, and respiratory sites[8-9], as well as for the detection of CRE[10]. Also, eSwab® is considered the gold standard method by the CDC-Atlanta for collecting samples for *C. auris* and subsequent determination of antibiotic resistance patterns. Samples collected in eSwab® can be directly struck on selective plates and used as inoculum for enrichment broth.

eSwab® is the standard device for collecting *Streptococcus agalatie* (Strep B or GBS) in pregnant women[11-13] from samples from the anogenital area. The eSwab® ensures a long preservation time and easily allows the inoculum into selective pre-enrichment broth.

The eSwab® is also perfect for collecting and preserving samples for *Streptococcus pyogenes* (Strep A or GAS) to detect the leading cause of

bacterial pharyngitis and subsequent detection of antibiotic resistance[14].

eSwab® has been proven extremely powerful in preserving the vitality of different types of fungi and yeast[15]. In this paper, the authors tested 19 isolates consisting of five yeast reference isolates (*Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida guilliermondii* ATCC 6260, *Candida glabrata* ATCC 66032, and *Cryptococcus neoformans* ATCC 66031), three reference dermatophytes (*Trichophyton mentagrophytes* ATCC 9533, *Trichophyton tonsurans* ATCC 28942, and *Trichophyton rubrum* ATCC 28188), five opportunistic hyaline molds (*Aspergillus niger*, *Aspergillus fumigatus, Lecythophora sp.*, *Fusarium solani*, and *Trichosporon sp.*), three Zygomycetes (*Lichtheimia corymbifera*, *Mucor circinelloides*, and *Rhizopus microsporus*), and three threedematiaceous molds (*Curvularia clavata*, *Phialophora americana*, and *Alternaria alternata*). All the species were successfully recovered from the eSwab®. *Candida auris* was also successfully recovered and transported in eSwab® from the armpit and inguinal swabs.[16]

eSwab® is considered an excellent collection device for testing women's health syndromes and sexually transmitted diseases from vaginal sites in women, as well as from urethral sites from both women and male samples. *Trichomonas vaginalis* has been proven to be successfully recovered from eSwab®[17]. Among the different pathogens in this class, *Neisseria gonorrhea* (NG) is one of the most concerning ones, especially because of the rise of antibiotic resistance in this pathogen. For a good epidemiological screening, the culturing of the pathogens is fundamental; thus, the vitality preservation in the collected sample is paramount. eSwab® was proven to preserve the vitality of NG[17-19a] efficiently.

FecalSwab®

A critical diagnostic field for microbiology is the detection of gastrointestinal pathogens (GI). Infective gastroenteritis can be caused by different etiological causes: microorganisms, viruses, bacteria, and parasites can all cause acute or chronic diarrhea. In the case of bacterial pathogens detection in stool, if the analysis is postponed after two hours from the sample collection (which is usually the case), the sample must be placed in a Cary-Blair-based preservative solution[19b]. For this purpose, Copan developed the FecalSwab®, a modified Cary-Blair medium specifically designed to preserve the vitality of entomopathogens at room temperature for up to 48 hours or up to 72 hours at 2-8 °C. The improvements of moving from native stool to FecalSwab® are multiple: higher sample stability, cleanliness, readiness of use, flexibility, and automatability. FecalSwab® can be used to preserve either native stool or rectal swabs and can be used for multiple serial or parallel analyses with different technologies.

Using FecalSwab® over an Amies or Stuart-based collection device is important because stool samples are extremely rich in commensal microorganisms, which may overgrow in a standard transport medium[20].

The use of FecalSwab® has been proven to double the Campylobacter recovery rate in clinical settings compared to native stool[21]. Campylobacter is probably one of the most fragile and faint bacteria causing enteritis, and the use of the FecalSwab® helps dramatically its recovery and, thus, diagnosis.

FecalSwab® was extensively evaluated also with automation platforms for plate streaking and incubation[22], as well as with non-human stool veterinary samples[23-24]. Very interesting studies successfully evaluated FecalSwab® ability to isolate *Helicobacter spp* species from stool[25] and microbiome samples[26-27].

Together with standard gastroenteritis diagnostics, the other most important field of application for the FecalSwab® is the preservation of rectal swabs for AMR screening, especially for ESBL, CRE, and VRE. The detection and monitoring of these resentences were evaluated by both cultural phenotypic assay and molecular approaches[28-39].

C. difficile infection (CDI) is among the most important healthcare-acquired infections (HAI). The high mortality associated with CDI, the high risk of hospital outbreaks[19], and the intrinsic complexity of *C. diff* diagnostics, stress, and even more, the need for an efficient preanalytical part.

FecalSwab® preserved stool was efficiently used to detect GDH and Toxin A and B[40-41].

UTM®

Nowadays, most of the diagnostics for viral infections are done by molecular assays or antigen tests. Despite this, the possibility of preserving the structure of the viruses remains paramount for allowing virus isolation for research, epidemiology, and clinical research. If the design of a transport media for preserving bacterial vitality recovery is complex, the creation of a proper viral transport media is even more critical as it must ensure that: 1) the viral capsid must stay intact; 2) the media must avoid bacterial and fungal growth 3) the media must be noncytotoxic to allow viral culture.
This is why only very few media can be considered "real" viral transport media, and Copan UTM can definitively be considered the gold standard. Over 200 publications used it for the recovery and later cultivation of multiple types of viruses, from respiratory to dermal and fecal.

MOLECULAR DIAGNOSTICS

The adoption of molecular biology by virology and microbiology labs is now a standard. In the early molecular days, these techniques required high skills and experience, dedicated areas for sample and reagent preparation, and experience in results interpretation; nowadays, with the latest automatic platform, molecular techniques are undoubtedly easier and more consistent than striking a plate. From small POC instruments to larger automated platforms that exploit different technologies (qPCR, LAMP, NASBA), nowadays, the offer is extremely high and differentiated. After the COVID-19 pandemic, even NGS and other sequencing techniques are becoming more present in diagnostics laboratories.
While for some pathogens, molecular assays are now practically the only choice (CT, NG, HPV…), for other pathogens, the option to go molecular or not can be driven by different factors like price, speed or reporting, or number of tests to run. Anyhow, the coexistence of molecular assays and classic microbiology techniques shall not be seen as a dichotomy but as a combination able to improve modern laboratories' results and supply complementary info. Also here, having the right sample quality is again paramount.
A good quality sample for molecular biology is when the nucleic acids (DNA and/or RNA) are preserved and not degraded. This may be achieved by preserving the cell's structure containing the nucleic acid, using active components that stabilize the DNA/RNA structure, or inhibiting the action of RNase and DNase.
In Copan's LBM range, all the transport media are compatible with molecular biology assays, even with different specificity.

eSwab®

Copan eSwab® is validated to preserve the DNA for up to 5 days at room temperature. Even if the main intended use of eSwab® is preserving bacterial vitality, the flexibility of performing molecular biology on the same sample allows for standardized sample collection, with all the advantages arising from this.
eSwab® was successfully tested and validated for the detection of Strep A[42], allowing the flexibility of molecular screening and culture reflex confirmation test and AST for the detection of Group B streptococcus[43], which allows standardizing on just one collection device both flow of patients that undergo the screening between the 35 to the 37 weeks of pregnancy by culture, and the ones tested in emergency during the labor by molecular.
eSwab® was successfully used with different molecular assays for the detection of *Chlamydia trachomatis* and of Neisseria gonorrhea (NG)[45-46], allowing also, in this case, the subsequential NG culture for AST. eSwab® can be used for the collection of samples for the molecular detection of women's health syndromes, like vaginosis and vaginitis syndromic panels[44].
As mentioned in the previous chapter on using eSwab® for resistant pathogens by culture, the same approach can be done by molecular assays. eSwab® is currently used to detect the genetic determinants of MRSA, CRE, and VRE[45-47]. Also, in this case, eSwab® allows the standardization of the collection part while leaving the freedom to

adapt the detection technology according to the case.

FecalSwab®

One of the fastest-growing fields where molecular biology plays a more critical role is the detection of gastrointestinal pathogens. MDx allows fast and comprehensive screening of multiple pathogens, including viruses, bacteria, and parasites. This is very important to preserve the vitality of the bacteria eventually present in the sample and to allow the culture and isolation of positive samples for bacteria.

In this landscape, FecalSwab® plays a crucial role. FecalSwab® is perfect for collecting stool samples for PCR screening of different bacteria, viruses, and parasites with different multiplex assays[48-55]. Typically, *C. difficile* diagnostics is managed out of the standard GI flow, and FecalSwab® has been proven to be highly efficient for collecting samples and preserving stools and compatible with the primary molecular assays available on the market[56-60].

FecalSwab® is extremely well-fitted to preserve samples intended for genetic determinants of antibiotic resistances, especially ESBL, CRE, and VRE from rectal swab[28-39].

A final mention of the FecalSwab® molecular capabilities is the detection of gastroenteritis pathogens from rectal swabs instead of stool 61-[63]. Different studies indeed demonstrated that the performances of the two types of samples are comparable. Using rectal swabs is highly advantageous for specific populations (newborns, children, and elderly) where stool collection can be problematic. In contrast, the collection of a rectal swab is fast and immediate, allowing prompt results delivery for a faster treatment response.

UTM®

When clinical necessity is the detection of viruses, the most known product is UTM®. Even if the UTM®'s original intended use is to preserve the viral integrity, most of the samples collected in UTM® are analyzed by NAAT or by antigen assay.

UTM® is routinely used for collecting samples for the detection of respiratory viruses (from either the upper or the lower respiratory tract), STI viruses (HSV 1&2 from blister and sores, monkeypox virus), and bacteria (*Chlamydia spp*., *Mycoplasma spp.*, *Ureoplasma spp.*), conjunctivitis viruses, and stool samples.

CONCLUSIONS

Liquid-Based Microbiology, together with the FLOQSwabs®, represented the most important improvement in the microbiology preanalytical phase of the last 100 years. The Copan's LBM line has now been used by thousands of laboratories worldwide, in millions of pieces per year. The quality and flexibility of the Copan's LBM allow one to find the right product for the application or pathogen you need to detect.

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