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Chromogenic Agar Media in the Clinical, Food, and Environmental Testing Arenas, Part II

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Abstract

In Part I of this two-part article, the author discussed two of the original product formulations of chromogenic agar medium used in the clinical microbiology laboratory for the recovery of *Candida* spp. and *Enterobacteriaceae*. CHROMagar Candida and CHROMagar Orientation media have been shown to have great value in the rapid recovery and identification of the various *Candida* yeast species and routine gram-negative bacilli from patient clinical specimens. In Part II, the article continues the discussion of chromogenic agar medium for the specific recovery of bacteria that are identifiable to the genus and species levels, notably, perhaps one of the most intensely sought after microorganisms of the last two decades – methicillin-resistant *Staphylococcus aureus*. Additionally, the article discusses further application of the chromogenic agar medium for environmental recovery and identification from soils, waters, and various food sources. Lastly, the article discusses the performance expectations for chromogenic agar medium by implementation of verification and validation procedures in the clinical microbiology laboratory.

Use of CAM for the Genus or Species Level Identification of Specific Microorganisms in Clinical Specimens

Salmonella spp. detection

Soon after the appearance of the initial reports of the utility of chromogenic agar media (CAM) for the detection of *Candida* species and members of the *Enterobacteriaceae*, a report by Gaillot et al. (16) indicated that CHROMagar Salmonella (CS) (CHROMagar Microbiology, Paris, France) was superior to Hektoen Enteric Agar (HEA) (Becton Dickinson, Le Pont-de-Claix, France) for the recovery of *Salmonella* spp., both in specificity (88.9% versus 78.5%) and in sensitivity (95% versus 80%). Further, Maddocks et al. (17) examined over 500 consecutive stool specimens and compared CS (Dutec Diagnostics, Croydon, New South Wales, Australia) to HEA, xylose-lysine-desoxycholate (XLD), and *Salmonella-Shigella* (SS) (Oxoid Australia, Heidelberg, Victoria, Australia) agars but concentrated on the combination of XLD and SS agar media compared to CS and determined that the specificity for CS was 83% versus 55% for the other two media. Although they cited the cost as a negative factor for inclusion of the CAM in the agar plate regimen for stool specimens, they found that it was offset by the substantial savings incurred in labor and the use of other consumables and reagents related to vast reduction in false-positive isolate detections and identification testing necessary to establish the accuracy of potential *Salmonella* spp.

Staphylococcus aureus detection

The greatest number of articles published for a specific pathogen detected using a CAM is for *Staphylococcus aureus* and its far more resistant form, methicillin-resistant *S. aureus* (MRSA). Gaillot et al. (18) and Carricajo et al. (19) reported on the utility of the new medium CHROMagar Staph aureus (CSA) (both articles indicate the source of the CSA as CHROMagar Microbiology). Gaillot conducted both analytical and clinical surveys with the medium compared to 5% horse blood agar (HBA). There were 2,000 consecutive specimens, from which 310 *S. aureus* strains were isolated and identified from one or the other media after testing coagulase positive. Of the 310 *S. aureus* isolates, 296 were recovered on CSA (sensitivity, 95.5%), and 254 were recovered from 5% HBA (catalase positive and latex positive; sensitivity, 81.9%). Specificity was high but equal

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for both media. In Carricajo's study, CSA was compared to Columbia agar medium supplemented with 5% horse blood. A total of 775 clinical isolates were studied, from which 267 *S. aureus* isolates were recovered from multiple specimen sites: 263 grew on CSA (sensitivity, 98.5%), and 243 grew on the conventional medium (sensitivity, 97%). Both authors stressed the efficiency and optimization of resources by use of CSA medium.

Kluytmans et al. (20) compared CSA (CHROMagar Microbiology) and oxacillin resistance screening agar base (ORSAB) (Oxoid Ltd., Basingstoke, United Kingdom) to determine the absence or presence of MRSA. CSA was tested unsupplemented with antimicrobials and supplemented with 4 μg/ml of oxacillin and then compared to ORSAB medium, unsupplemented and supplemented with polymixin and oxacillin at 2 μg/ml. A mixture of 358 methicillin-susceptible *S. aureus* (MSSA), and 266 MRSA strains and 516 coagulase-negative staphylococci were used. The sensitivities of the two media were high in the detection of MSSA and MRSA when both media were unsupplemented. However, the sensitivities were far lower when both media were supplemented with oxacillin. The authors concluded that the unsupplemented CSA was more accurate than the unsupplemented ORSAB medium after 24 hours of incubation in detecting *S. aureus*, because the specificity of the CAM was far higher. However, ORSAB supplemented with antibiotics had greater utility in detecting MRSA at 24 hours than the CAM.

Flayhart et al. (21) used respiratory specimens from cystic fibrosis (CF) patients and nasal surveillance cultures in their comparison of CSA (BD Diagnostics) with tryptic soy agar with 5% sheep blood agar and mannitol-salt agar (MSA) media (BD Diagnostics). There were 679 consecutive clinical specimens studied, with 98% correlation in recovery of 192 *S. aureus* isolates on both media from all specimens. However, the CSA recovered slightly greater numbers of *S. aureus* strains than the conventional medium, i.e., five more *S. aureus* strains from nasal surveillance culture, five more *S. aureus* strains from CF throat cultures, and two more *S. aureus* strains from CF respiratory specimens. The authors concluded that the medium had the potential to identify health care workers and patients who might be colonized with MRSA in the hospital environment.

Diederen et al. (22) reported on CHROMagar MRSA (CMRSA) (CHROMagar Microbiology) using a combination of well-defined MSSA (241 strains) and MRSA (216 strains) isolates representing a broad section of specific type strains. The sensitivity of CMRSA was 95.4% at 24 h and 100% after 48 h of incubation. Specificity was 100% at both 24 h and 48 h. Hedin and Fang (23) reported on their placement of a cefoxitin disc (10 μg) onto *S. aureus* ID agar medium (SAID) (BioMérieux) in comparison with CMRSA (CHROMagar Microbiology), 5% HBA, and MSA (both supplemented with 4 μg/ml of cefoxitin) to measure the detection of MRSA. Using a standard reference method prescribed by the Swedish Reference Group for Antibiotics using Isosensitest agar (Oxoid Ltd., Basingstoke, United Kingdom), the investigators noted identical results identifying MRSA via SAID with the 10-μg cefoxitin disc and CMRSA. The investigators suggested that a cefoxitin disc could be placed on CAM not containing antibiotics, as they noted 10% of known MRSA isolates failed to grow on CSA supplemented with 4.0 μg/ml of either oxacillin or cefoxitin.

Flayhart et al. (24) reported on the results of a surveillance study for the presence of MRSA in a four-site multicenter evaluation. A highly descriptive schematic presentation of the study design was presented, in which CMRSA medium was compared to tryptic soy agar with 5% sheep blood. The data showed more recovery of MRSA on CMRSA than on conventional media and that there was no breakthrough growth of MSSA on CMRSA. In addition, the authors compared the detection of MRSA on CMRSA against five conventional susceptibility-testing methods and found excellent agreement of the CMRSA results with those of conventional methods, on average, at around 94%.

Stoakes et al. (25) examined 2,125 consecutive nasal and perineal swabs for the presence of MRSA using four agar media: MSA-oxacillin (6 mg/liter) (MSA-OXA) (Oxoid, Ottawa, Canada), MSA-cefoxitin (MSA-CFOX) (Oxoid, Ottawa, Canada), CMRSA (Becton Dickinson), and MRSA*Select* (MRSAS) (Marnes la Coquette, France). Sixty-one MRSA isolates were recovered. The reference test standard was multiplex PCR amplification of the *nuc*, *femB*, and *mecA* genes. The CAM performed much better than either of the MSA agars with supplemented antimicrobials both in the number of MRSA strains detected and the vastly lower numbers of false-positive colony isolates encountered. Although MSA-CFOX showed 99.1% sensitivity, it only had a 26.5% positive predictive value (PPV). The two CAM had high sensitivities (CMRSA, 82.9%, and MRSAS, 97.3%), specificities above 99%, and excellent PPV, ranging between 84.4% and 97.3%, respectively. Compernolle et al. (26) examined three agar media in a similar study, Oxacillin Resistance Screening Agar (ORSA,

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Oxoid Ltd., Basingstoke, England) with and without enrichment in tryptic soy broth (TSB), CMRSA (Becton Dickinson Sparks, MD), and MRSA ID (BioMérieux). Three-hundred sixty-six specimens from a variety of body sites were examined. Thirty MRSA strains were recovered. A variety of reference test methods were employed in the investigational design, including a tube coagulase test, Pastorex Staph-Plus Latex agglutination test, PCR testing for *mecA* and *nuc*, and gram stain. The results showed that the CAM performed better than ORSA with or without enrichment broth. The numbers of true-positive MRSA isolates recovered were relatively equal, but the numbers of false-positive isolates encountered were vastly higher on the ORSA agar medium (with or without enrichment in TSB), leading to far lower specificities.

Van Hal et al. (27) studied the performance of two commercial molecular methods for the detection of MRSA challenged against three CAM selective for MRSA serving as the reference test standard. The CAM were MRSA ID (BioMérieux), MRSA*Select* (Bio-Rad Laboratories, Hercules, CA), and CMRSA (CHROMagar Microbiology), and the molecular methods included the IDI-MRSA assay (Infecto Diagnostics Inc., Sainte-Foy, Quebec, Canada) with a Smart Cycler II rapid DNA amplification system (Cepheid, Sunnyvale, CA) and the GenoType MRSA Direct (Hain Lifescience, Nehren, Germany). Two-hundred and five patient specimens obtained from nasal, groin, and axilla body sites were examined and 78 true-positive MRSA isolates were identified. The authors noted that results obtained with molecular methods were more rapid than traditional overnight incubation and had greater potential to limit the spread of MRSA in hospital environments, as the turnaround times to the results were faster. The IDI-MRSA molecular method showed the greatest sensitivity at 24 h compared to GenoType MRSA and the CAM for identification of MRSA at 24 h and 48 h. The specificities of the two molecular methods were equivalent to the specificities of the CAM at 24 h, but an additional 24 h of incubation led to decreased specificities for the CAM. The authors noted a lower recovery rate on CAM than previously reported and attributed the differences to the "inoculum effect" of having only one swab to inoculate three selective agars. Adjusting for the inoculum effect by eliminating non-unanimous positive findings from the three selective media still did not improve the overall sensitivity. The authors heralded the short turnaround time to a positive finding but lamented the overall expense of the molecular methods compared to CAM or conventional-medium approaches.

Boyce and Havill (28) conducted a smaller study using CMRSA and the BD GeneOhm MRSA real-time PCR system (both BD Diagnostics). They examined 286 nasal swab specimens and recovered 69 true-positive MRSA isolates. They noted that the PCR method was more sensitive, had a faster turnaround time of 13 hours, and led to faster isolation of MRSA-colonized patients but that CMRSA similarly detected a majority of the positive MRSA results within a 24-hour time frame. Cost considerations, staffing, and operational testing were also analyzed and discussed. Farley et al. (29) conducted a similar study with CMRSA and the BD GenOhm MRSA assay (both BD Diagnostics, Sparks, MD). Six-hundred and two patient nasal swabs were examined, and 123 positive results were obtained via the PCR method. However, there were 56 discrepant results between the CMRSA growth and the PCR findings. Additional investigation determined that the *mecA* gene was missing in a proportion of the PCR-positive specimens. The authors discussed the possibility that use of a sole molecular method may be diagnostically unreliable.

Pape et al. (30) used CMRSA (BD Diagnostics) medium for the direct subculture of positive blood cultures showing gram-positive cocci in clusters on Gram stain. This approach showed a sensitivity of 97.6% at 18 to 24 h and 100% by 48 h and was 99.9% specific.

Additional CAM formulations

Church et al. (31) reported on the performance of BBL CHROMagarO157 (CO157) compared to Sorbitol-MacConkey medium (both BD Diagnostics) for the isolation of *Escherichia coli* O157. In a survey of over 3,000 consecutive stool specimens collected over the summer months, 27 isolates were identified as *E. coli* O157. The authors showed that CO157 medium provided savings in terms of reagents, labor, and time in reporting a true-positive isolate because the CO157 eliminated false-positive colony picks that would have necessitated additional work-up with routine Sorbitol-MacConkey medium.

Samra et al. (32) designed a study using CHROMagar KPC (CHROMagar Microbiology) (KPC, *Klebsiella pneumoniae* carbapenemase) to identify isolates of *Enterobacteriaceae* capable of producing carbapenemase compared to a reference method using MacConkey agar onto which were placed carbapenem discs including imipenem (10 μg), meropenem (10 μg), and ertapenem (10 μg). There were 122 carbapenem-resistant *K. pneumoniae* isolates tested, resulting in sensitivity/specificity of 100%/98.4% using CHROMagar KPC versus 92.7%/95.9% for MacConkey agar. PCR was used to resolve discrepant results.

CHROMagar Acinetobacter (CA) (CHROMagar Microbiology) was reported as a useful screening medium to test for the carriage of *Acinetobacter baumannii* in the stool or perineal specimens obtained from critically ill patients by Gordon and Wareham (33). PCR was used as the comparative method and detected 3 specimens from 103 patient specimens that were negative by CA. However, PCR failed to detect 7 specimens that were previously shown to be positive using CA. The authors concluded that CA was useful but acknowledged that their specimen survey was limited by the small numbers tested.

Use of CAM for the Species-Level Identification of Specific Microorganisms from Water, Soil, and Environmental Specimens

Water and soil

Alonso et al. (34) used CHROMagarECC (CECC) (CHROMagar Microbiology) to monitor and measure the presence of *E. coli* and other fecal coliforms in river and marine waters after filtration of the water samples. Ishii et al. (35) used CECC for confirmation of *E. coli* isolated from soil near the Lake Superior watershed. Ishii et al. (36) went further to describe the isolation of environmental *Salmonella* spp., Shiga toxin-producing *E. coli* (STEC), *Shigella* spp., and *Campylobacter* spp. from algal blooms of *Cladophora* spp. abundant on the Lake Michigan shore-

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line. These environmental specimens were subjected to extensive preliminary isolation methods, but once presumptive isolates of *Salmonella* and *E. coli* were growing, they were subcultured to Rambach agar and CO157 agar medium plates (both CHROMagar Microbiology), respectively. Hamner et al. (37) used CO157 (CHROMagar Microbiology) as a final subculture screening medium for Ganges River water samples inoculated originally onto MacConkey agar for isolation of potential coliforms, followed by transfer of isolates to nutrient agar. Levin-Edens et al. (38) reported on the recovery and enumeration of MRSA isolated from marine and freshwater specimens by the use of MRSA*Select* medium (Bio-Rad). Rogers et al. (39) reported on the use of CS and CO157 in an assessment for the isolation of the respective pathogens from spiking studies of soil enriched with manure.

Environmental

Keen et al. (40) reported on the isolation of *Salmonella* and STEC O157 from zoo animals using Rambach agar (CHROMagar Microbiology) and CO157. Nearly 1,000 specimens from a wide variety of zoo animals were obtained. These animals were part of interactive human-animal contact exhibitions, such as animal petting areas. Five *Salmonella* spp. (0.6% prevalence) and 1 STEC O157 strain (0.1% prevalence) were recovered from 871 specimens obtained from the domesticated animal exhibits at 36 facilities across the nation.

Zapka et al. (41) used CO media (BD Diagnostics) as a qualitative confirmation of results observed with MacConkey agar medium in an experiment examining gram-negative isolate contamination of hands from "seeded" public bulk soap refillable dispensers. The purpose of the experiment was to assess the probable transmission of the acquired contaminants on the hands to secondary surfaces. Although data for the CO was not presented, the selectivity for recovery of the "seeded" contaminants was presumably similar for the two agar media.

Seafood, poultry, cattle, pigs, and produce

CAM has become indispensable to the primary isolation of specific microorganisms in rapid testing regimens in these commercial food collection and preparation areas. Blanco-Abad et al. (42) reported on the use of a direct-PCR method, CHROMagar Vibrio (CV) (CHROMagar Microbiology) and thiosulfate-citrate-bile-sucrose (TCBS) agar for the recovery of *Vibrio parahaemolyticus* from 259 marine samples, including zooplankton, mussels, and environmental seawater. In this absence/presence approach, CV clearly outperformed the TCBS agar, but the direct-PCR method outperformed both of the agar media, detecting *V. parahaemolyticus* in 23.6% versus 11.2% of samples. Mahmud et al. (43) conducted a similar study with the same media and PCR techniques, but the specimens tested were from netted Tilapia fish grown in aquaculture and associated water samples. Slightly higher rates for recovery of *V. vulnificus* were reported through use of the CV compared to TCBS, although specific data were not stated. Roque et al. (44) reported on the primary isolation of *V. parahaemolyticus* on CV (CHROMagar Microbiology) following alkaline-peptone-water broth enrichment from nearly 800 marine specimens of mollusks, including oysters, mussels, and clams. Two-hundred and thirty-one isolates were recovered from CV, 154 of which were found through PCR analysis to contain the *tl* gene, a species marker for *V. parahaemolyticus*. Thirty-two of these 154 were found to be toxigenic by the determination of the presence of the *tdh* and *trh* genes. Ward and Bej (45) used CV (DRG International) in a seeded oyster experiment by inoculation of serial dilutions of overnight-enriched samples to determine the viability of the cultures and total plate counts. Isolates were then analyzed for the presence of the *tlh, trh*, and *tdh* genes, as well as a DNA sequence open reading frame 8 (ORF8), to detect strains of the recently emerged, increasingly virulent pandemic strain of *V. parahaemolyticus* O3:K6. Finally, Hara-Kudo et al. (46) used CV (CHROMagar Microbiology) to determine the total numbers of *V. parahaemolyticus* isolates from a wide-ranging variety of consumable seafood products from Japanese fish markets. *V. parahaemolyticus* was confirmed through inoculation into triple-sugar iron (TSI) medium (Eiken Co., Tokyo, Japan), nutrient broth (Difco), and nutrient broth supplemented with 8% NaCl. Strains showing and alkaline slant and acid butt reactions in TSI and growth in nutrient broth with 8% NaCl but no growth in broth without the salt concentration were identified as *V. parahaemolyticus.* The presence of the *tdh* gene was determined through PCR analysis.

Chicken, cattle, and pigs

Pleydell et al. (47) reported on the use of CECC in the recovery of presumptive *E. coli* from the feces of organic broiler chickens. The recovered *E. coli* were classified as ampicillin resistant or sensitive by their ability to grow after the incorporation of 8 μg/mL ampicillin into the CECC agar medium. Keen et al. (48) utilized CO157 (manufacturer and location not given) for primary isolation of STEC from numerous body sites in cattle during necropsy. Arthur et al. (49) utilized CO157 (DRG International) and three other agar media for the primary isolation and quantification of STEC O157 from cattle hides and the surrounding holding-pen environment. Finally, Horton et al. (50) utilized CECC and CHROMagar CTX (M-Tech Diagnostics Ltd., United Kingdom) for primary isolation and determination of the prevalence of extended-spectrum β-lactamase production in *E. coli* from the cecal contents of chickens, and the feces of cattle and pigs.

Produce

Massive outbreaks of food-borne illnesses have occurred within the last 5 years across North America, Europe, and Russia. The vegetable and food varieties incriminated include spinach, bean sprouts, tomatoes, peppers, salsa, and guacamole. The CAM have been shown to be useful in the recovery of various human pathogens and food spoilage microorganisms from these and other produce items. Tornai-Lehoczki et al. (51) used CHROMagar Candida (CC) (CHROMagar Microbiology) to recover and identify 93 yeast strains representing 33 species and 39 fresh yeast isolates recovered from mayonnaise-based salads in an examination of salad spoilage contaminants. Their conclusion was that CC facilitates the detection of mixed yeast species from different kinds of foods on a single plate in a mechanism that is practical, efficient, and rapid. Similarly, Gorski et al. (52) used CS to determine the

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prevalence of *Salmonella* spp. in a wide-ranging survey that included environmental, wildlife, and produce samples. Over 2,400 samples were examined, and 55 were positive for the presence of *Salmonella* spp., yet none of the samples from pre-harvest lettuce and spinach were found to be positive. Finally, Deng et al. (53) utilized CO157 (DRG International) to recover and isolate eluted *E. coli* O157 bacteria from the seeded leaves of spinach in an investigation to determine the survival rates of the microorganism in the presence of chlorine.

CLSI Input on the Use of Chromogenic Agars

In January 2011, the Clinical Laboratory Standards Institute (CLSI) published document X07-R, entitled *Surveillance for Methicillin-Resistant Staphylococcus aureus: Principles, Practices, and Challenges; A Report* (54). The committee members commented about the use of CAM in the clinical microbiology laboratory. Notably, they indicated in section 6.3.3, Quality Control, that fresh subcultures of *S. aureus* should be kept on hand, as there might be a need to perform quality control (QC) more frequently with CAM than with other routine agar media. Secondly and importantly, the committee drew attention to the fact that CAM, despite having varying concentrations of inhibitory antimicrobials as part of the formulation, were not to be used as a "quasi-substitute" mechanism for susceptibility testing or for an immediate susceptibility interpretation based on growth of resistant *S. aureus* on the media.

Section 8.1.2, entitled Urgency, contrasted the time to report a positive finding for MRSA using real-time PCR molecular-based methods. PCR methods were reported to be 98% sensitive and to take less than 2 h to complete a report. In contrast, directly inoculated CAM were reported generally to be 80% sensitive after 24 h of growth. However, microorganisms that were enriched in broth and subcultured onto CAM were reported generally to be 90% sensitive after 48 h of growth. However, colony isolates identified as MRSA growing on most of the commercial CAM formulations for MRSA detection currently approved by the FDA are considered to be a confirmatory test result after 24 h of growth. Check the manufacturer's package insert for claims.

The CLSI document generically lists five manufacturers' formulations for their CAM formulation, including references to the "chromogens," colors produced, and "inhibitors" placed in the medium mixture, but without specifics. The reader is encouraged to refer to the CLSI document and the appendixes for details.

Verification Procedure of CAM for the Clinical Microbiology Laboratory

Incorporating CAM into the routine agar plate inoculation of clinical specimens is relatively easy. In accordance with the guidance provided in Cumitech 31A (55), a laboratory must verify the manufacturer's performance specifications for a new product. Although routine agar media are generally exempted from verification checks, more complex and fastidious media are not exempt. It is scientifically sound practice to establish the performance criteria through initial verification followed by a regularly scheduled QC test.

Performance specifications are defined as "accuracy (i.e., agreement), precision (i.e., reproducibility), reportable range (i.e., the final result), and the reference range (i.e., the normal value reported)."

Accuracy is measured by the concurrent testing of the new product alongside the laboratory's routine reference method. For example, to verify a CAM for MRSA for anterior nasal swab specimens, an approach might include the use of a double swab that was rotated five times within each nostril. One swab would be used to inoculate a standard medium, and the second would be used to inoculate the CAM for MRSA. Following the appropriate incubation period in accordance with the manufacturer's instructions, the colonial growth on each plate would be assessed with tests such as the Gram stain, coagulase, and a disk test for cefoxitin resistance. The results for the routine medium should be the same as the visual assessment obtained from the CAM for MRSA. The maximum sample size to be tested should be the laboratory director's decision but should reflect a statistically valid sampling based on the known prevalence of the pathogen within the population.

Precision is the ability to obtain identical results for specific specimens regardless of when the specimens are inoculated during the verification process or by whom the specimens are inoculated. Using a CAM for MRSA as the example again, it would be reasonable for the laboratory director to create a panel of previously identified specimens that are positive and negative for the presence of MRSA. The specimens could originate from the daily clinical specimen load, or they could be prepared from stock isolates. Dual culture swabs facilitate this process. Using a previously inoculated specimen swab from earlier in the day, the medical technologist can employ the very same swab at a different time point during the day to inoculate another agar medium plate. Monitoring the results for identity would suffice. Similarly, having a second medical technologist re-inoculate the same specimen would also suffice. The laboratory director can make the decision as to the maximum specimen size to test.

The reportable test range is a comment similar to indicating the presence or absence of MRSA in the usual laboratory vernacular. The reportable reference range for clinical specimens should be a comment indicating the absence of MRSA, because that is the expectation. The reportable reference range for analytical specimens would be a comment similar to the presence or absence of MRSA.

All records of the verification process need to be stored and maintained for easy access should the laboratory be called upon to provide this proof during an inspection. Paper files are passé; electronic files are much more easily retrievable, they can be a part of most laboratory information system QC records, or they can be created and stored easily in Excel files.

Validation Procedure for CAM in the Clinical Microbiology Laboratory

Many laboratories face the prospect or necessity of testing specimens in ways that were not envisioned by manufacturers. A simple anterior nasal swab may be suitable as a screen for the pres-

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ence or absence of MRSA, but ongoing laboratory investigations and reports have indicated that there are other body sites from which MRSA could be recovered (56). Indeed, when the product or the specimen to be tested has not been previously granted clearance by the FDA it is imperative that the laboratory conduct a validation procedure to establish that the product or the specimen produces results that are in accordance with the laboratory's expectations for the product or the specimen. For example, a report by Wendt et al. (56) indicated that MRSA can be recovered from many other body sites, including the axillary and inguinal areas, wounds, stool, and blood. Thus, in the event that these specimen types are submitted as acceptable specimens for MRSA detection, the laboratory should perform a validation procedure with the specimens using the CAM of their choice. Similarly, if the laboratory has reason to modify an FDA-cleared product's procedure, e.g., the length of incubation time, the process must be validated. Validation exercises should involve the use of known specimens. The maximum number of specimens suggested for study in a validation procedure should be the laboratory director's decision, but again, the total number of specimens tested or the total amount of time a procedural change is investigated needs to be adequate to withstand the scrutiny of statistical analysis. Validation records need to be on file until the method is retired.

Manufacturers and their CAM Product Line-Ups

An Internet search of well-known American and European medium manufacturers was conducted to produce a table listing the variety of CAM products. The offerings reach beyond the clinical microbiology laboratory into commercial arenas, where the specified pathogens can serve as outbreak sources that are food borne, waterborne, or related to environmental contamination. See Table 1 in Part I of this article for a list of available products by manufacturer. Formulations may vary between manufacturers. Carefully check the manufacturer's package insert for specific claims and uses. (Manufacturers' product offerings often vary. The author does not claim that the list is thorough. Any omission of a manufacturer or product from the list is purely unintentional. The author offers apologies to any manufacturer whose products are not included in the list.)

Conclusion

The classic approach for detection, isolation, and identification of a specific microorganism in the clinical microbiology laboratory has been the observation of a single colony with unique phenotypic clues that hint at its identity, followed by a testing regimen that pinpoints the name of the microorganism. The testing regimen can be a series of simple, rapid tests or a more complex procedure using an instrument-based identification. The process can take many days. CAM have substantially reduced the amount of time, effort, resources, and funding that was expended in the past toward this goal. CAM are playing a significant role in the laboratory diagnosis of infectious disease pathogens and, for all intents and purposes, will continue to facilitate an easy-to-use and reliable identification method in the clinical microbiology laboratory.

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