**FISH versus aCGH**

Clinical laboratory genomics is an important issue for your readers since it is likely to change the landscape of laboratory testing for many medical conditions in coming years. Drs. Billings and Brown have presented an overview of advances in this rapidly changing and growing discipline in a single article, an admirable objective. In prognostications about future laboratory testing, it is unfortunately too easy to become tantalized by technologies without considering the limitations of those very technologies. This does a disservice to your readers because the lack of balance implies that existing technologies are in some way less adequate. Some of your readers may be learning about these new technologies for the first time, so it is important that the information be presented in a responsible manner.

We are writing to correct some errors in the section of this article titled “Chromosome Imaging.” The paragraph on array comparative genomic hybridization (aCGH) on page 12 [December 2004] begins by stating that this technique is more “powerful” than FISH. The term “powerful” is inaccurate. aCGH is a highly multiplexed version of FISH (using often the same reagents), except that no information about chromosomal context of individual probes is provided. The article asserts that array technology will displace conventional cytogenetic analysis by eliminating “the need to culture cells or stain chromosomes.”

Conventional cytogenetics and FISH identify balanced translocations, which cause the majority of leukemias and solid tumors. aCGH cannot and will not provide this essential clinical information. Also, often many regions of the genome show amplification or loss of heterozygosity by aCGH. The complex presentation of such findings has been of limited value in determining correct diagnosis or optimal treatment regimen. Until reliable algorithms are developed, we must question the purpose of using genome-wide aCGH instead of a series of targeted FISH probes.

The genomic resolution of aCGH is approximately equivalent to chromosomal banding at present and, therefore, offers no advantage for a board-certified cytogeneticist. Finally, aCGH is “noisy” and difficult to interpret unequivocally, a fact that most proponents of this technique tend to downplay. The noise appears to be related either (1) to inconsistency in the quality of repetitive sequence-enriched DNA required for assay specificity or (2) to copy-number polymorphisms that are found in homologous chromosomes from the same and from different individuals. It is not clear at this point that it will be possible to adequately control for these sources of variation to develop reliable clinical tests. The authors suggest that an advantage of array technology is that assays can be repeated. This is not the actual reason for carrying out duplicate experiments. Because of the experimental variability of aCGH, it is absolutely necessary to perform duplicate experiments. FISH is more robust because it is internally replicated (i.e., multiple cells are individually scored within the same assay).

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Response from Drs. Matthew P. Brown, Paul R. Billings, and Peter Papenhausen of the Laboratory Corporation of America Holdings’ Center for Molecular Biology and Pathology, Research Triangle Park, NC:

We thank Drs. Rogan and Knoll for their interesting and thought-provoking reply to our article, which was intended to be a general survey of new technologies in use or on the horizon for clinical laboratories. We agree that our article did not compare older with newer methods; that was not our charge. In fact, we gave such an example when we discussed the use of IHC techniques for primary screening of HER-2 with reflex to the more costly but more specific and sensitive FISH methods for indeterminate results.

To reply more specifically to Drs. Rogan and Knoll’s objections, we did not state that aCGH was more powerful than FISH, rather that it was more powerful than conventional CGH.

**Letters to the editor**

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*MLO* welcomes letters to the editor. We ask that you include a phone number for verification. While we prefer to publish the writer’s name, we will publish a letter with “name withheld by request,” but our editorial staff must have the writer’s name confirmed for our files. *MLO* reserves the right to edit any letter for style and length.
We did not state that aCGH could detect balanced rearrangements, only that culturing and staining were not necessary. Clearly, aCGH is best used when dosage change is the primary target, as in most cytogenetically abnormal miscarriages or carcinomas rather than sarcomas, which often are caused by balanced gene fusions/translocations.

Drs. Ragan and Knoll state that “Often many regions of the genome show amplification or loss of heterozygosity by aCGH. The complex presentation of such findings has been of limited value in determining correct diagnosis or optimal treatment regime.” We would point out that this limitation is in large part due to the fact that ongoing studies of aCGH are only now being pooled. It is the potential of evaluating hundreds of genomic sites simultaneously without the need to culture cells that makes aCGH more powerful than conventional cytogenetics. Additionally, the resolution is well above that of chromosomal banding, and complex imbalance is immediately defined and generally corroborated by multiple linearly positioned BACs. BACs demonstrating polymorphisms are replaced by those not revealing such variations.

In conclusion, progress in the use of the technologies we noted in our manuscript may produce new and more precise information than previously available. Once these technologies are clinically validated, it is likely an improvement in the performance of clinical labs will result; aCGH may be such an example. The density of the platform should provide more information than can be derived from current conventional and FISH-based cytogenetic analyses. While we are not suggesting that aCGH will completely displace all utilization of conventional cytogenetic analysis, we do believe that a growing body of evidence indicates that this technique may provide crucial new information not delivered by older technologies in important clinical situations.

Antibiotic-removal devices defended

I wish to commend Colleen Gannon on a well-written article (“Responsible reporting in microbiology…” December 2004, p.18) describing many of the clinically relevant reporting practices which we employ and which I recommend to participants at my traveling workshops. However, I wish to comment on two of her statements regarding the use of antibiotic-removal devices in blood cultures.

Gannon’s statement that blood-culture antibiotic-removal devices confuse physicians is based on the presumption that antibiotic therapy is the only critical part of managing a patient with a life-threatening septicemia. Of equal importance in saving the patient’s life is how the focus of infection is addressed. In sepsis, what one does not [do] involving antibiotics is often more important than what antibiotic is used.

For example, if an Escherichia coli blood-culture isolate, recovered by a blood culture with an antibiotic-removal device, tests sensitive to cefazolin and the patient is receiving this antibiotic, this may mean the patient has an unsuspected leaking diverticulitis in need of surgical repair or, perhaps, needs to have a urinary tract catheter replaced or removed.

If the patient whose blood culture is growing vancomycin-susceptible Staphylococcus aureus and is being treated with vancomycin — an antibiotic removed by BACTEC resin bottles, but not FAN bottles — is failing vancomycin therapy, this may be secondary to an unsuspected intravenous catheter-associated infection or an inadequately drained wound. In short, if a patient has breakthrough bacteremia, ignorance is not bliss but, rather, can have severe or life-threatening consequences.

The author also stated that the use of antibiotic-removal blood-culture media may increase the rate of contaminated blood cultures. I am unaware of any published studies showing increased rates of contamination with the BACTEC resin media versus any standard media. However, her concern with respect to the FAN bottle is well founded. In addition to the reference she cited, there are three other studies showing increased rates of false-positive blood cultures associated with the use of the FAN media.

Despite the apparent contamination problem with the FAN bottle, the Weinstein reference cited by Gannon does show that, compared to the standard BacT/ALERT bottle, use of the FAN bottle does result in detection of considerably more cases of clinically significant bacteremia. Not only is this medically important, but not using the FAN bottle might decrease prospective payment septicemia reimbursements. Therefore, I would recommend that the users of the BacT/ALERT system routinely employ FAN bottles in every adult blood-culture set drawn.

To minimize the contaminated blood-culture problem in any blood-culture system, there are important measures that can be taken. One key is an effective blood-culture collection service in which not only proper techniques are discussed but [also] one in which an audiocassette tape by a respected physician is played, describing the medical dilemmas posed by false-positive blood cultures.

Another key is to use a separate alcohol swab to disinfect the blood-culture bottle top. If the same swab that is used to disinfect the venipuncture site and the palpation finger is used on the top of the blood-culture bottles, the resultant transfer of skin flora to the blood-culture bottle may give a false positive.

If nurses, physicians, or emergency room personnel are drawing blood cultures, having blood-culture procedure trays in which blood-culture bottles are packaged together with venipuncture-preparation supplies may also help. When people, other than laboratory-trained phlebotomists, draw blood cultures, are in a hurry, and cannot find venipuncture supplies, they may take shortcuts that increase contamination.

—Dennis L. Wegner, PhD
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Colleen Gannon’s reply to Dr. Wegner:

Thank you for your comments regarding my article. Blood cultures are among the most important laboratory tests performed in the diagnosis of serious infections. It is equally important to note that contaminated blood cultures are common, enormously costly (it would cost our institution an added $16,319 a year to use a blood bottle with an antibiotic-removal device with each blood-culture set), and frequently confusing to clinicians.

It does not seem practical to use an antibiotic-removal device for each blood culture drawn when the vast majority of cultures are obtained before administration of antibiotics. (We routinely utilize a blood-culture bottle containing an antibiotic-removal device on all infants and young children because frequently
they have been on a failed course of orally administered antibiotics before coming to the emergency department.)

In your example — if a cefazolin-susceptible *E. coli* isolate is recovered using an antibiotic-removal device in a patient receiving cefazolin, it might indicate an unsuspected leaking diverticulitis — this seems like an uncommon situation needing expert evaluation by the attending physician and an infectious-disease specialist, not to mention utilizing more precise diagnostic procedures, such as CT scan and angiography, in symptomatic individuals. I maintain that physicians would more likely determine that the antibiotic was not working.

Many hospitals and laboratories have noted an increasing proportion of blood-culture isolates that represent contamination compared with those in years past. This may be due to the newer continuously monitoring blood-culture systems that detect microorganisms present in low numbers more rapidly. In addition, broth medium formulations containing antibiotic-removal devices (e.g., BACTEC Plus Resin media and BacT/ALERT FN media) have been shown to improve detection of staphylococci, including coagulase-negative staphylococci, which often are contaminants.

Our institution conducted a statistical evaluation of blood-culture results obtained during a six-month period using bottles containing antibiotic-removal devices and compared it to blood cultures gathered over six months using standard blood-culture bottles without removal devices. Overall percentages of positive results for both systems were comparable, including percent contamination rates for each system (less than 3% overall).

**Medicine must be a “calling”**

I would like to respond to Dr. Block’s rebuttal of the *MLO* article, “Responsible reporting in microbiology: improving quality of care through better communication,” December 2004 [p. 18].

As a pathologist with specialty boards in microbiology, I would like to make some comments.

1. The fundamental issue and focus should be what is best for the patients.

A laboratory with appropriate physician oversight by pathologists develops policies at the direction of the pathologists. Before these policies are instituted, they are often sent to the medical staff for comment and necessary adjustment, depending on medical staff input. Ultimately, the final arbitrator is the director of the laboratory, who weighs all the concerns of [his/her] colleagues and current scientific information, and makes a final decision, which he/she feels is in the best interest of the patient. Thus, if the clinical director signs off on policies, the liability issue rests with the laboratory director and not the clinician. Furthermore, a clinician is not obligated or may recognize a unique circumstance in which he/she may decide an alternate treatment would be more appropriate. Sadly, many hospitals have de-emphasized the role of the laboratory pathologist in quality hospital care.

2. The laboratory provides information to a broad variety of medical caregivers. These include not only physicians with variable knowledge and skills but also nurse practitioners and physicians’ assistants. To state that the clinician should be aware of total Faustian knowledge in medicine is unrealistic. Therefore, any assistance a lab can give the clinician regarding the interpretation of a lab test can only benefit the patient.

3. Reading between the lines of Dr. Block’s letter, the real issue is the sorry situation that medicine is in today. Frivolous liability torts can destroy a physician’s [reputation] with hysterical front-page newspaper headlines. In addition, this issue ultimately hurts patient care. I do not know the answer to this dilemma; all I can say is that I am not God, and I can only strive to continue to do my best.

William Osler, the father of internal medicine and a philosopher, once said, “First, medicine must be a ‘calling.’” And that is why I went into medicine.