A Deeper Look at PRAS Media: Best Practices to Isolate and Grow Anaerobes Faster and More Reliably

While PRAS plates involve a slightly higher out-of-pocket expense, that cost is often dramatically outweighed by the benefits.

The demanding task of isolating and growing anaerobe microorganisms has become simplified and more accurate with the development of commercial PRAS media, and has achieved the status of Best Practice in evidence-based microbiology.

Today, clinical labs are increasingly adopting PRAS plates not only because they seek more reliable results that meet Best Practice criteria, but also because they are finding that manufactured PRAS plates are in a number of ways more reliable and economical.

"With PRAS media you get better growth than traditional non-PRAS media," says Timothy R. Cassity, Ph. D., Clinical Microbiologist at Southern Ohio Medical Center (Portsmouth, OH). "Anaerobes normally grow slowly, and some produce such small colonies that they are difficult to work with. Because the same anaerobes grow more quickly and abundantly on the commercial PRAS media, it is much easier to work with them."

In a Comparison Study of Anaerobic Culture Media conducted by Cassity this year, he compared OxyPRAS Plus Brucella agar (manufactured by Oxyrase, Mansfield, OH) and non-PRAS media he was using for the general culture of anaerobes. All plates were incubated in Anoxomat jars. The study found that the colonies on the OxyPRAS plates were about twice the size of those on standard TSA with blood and slightly larger than the ones on CDC anaerobic blood agar that was not reduced before use.

"Also, the study found the difference is more noticeable with slower growing anaerobes than with more rapidly growing ones, like Clostridium spp," Cassity notes. "Since the

growth on the OxyPRAS Plus Brucella agar was more luxuriant than the plates we had been using, we are replacing them with OxyPRAS Plus Brucella plates; including subculturing anaerobic isolates for identification on the Vitek 2 (microbial identification system)."

The PRAS difference

Isolating and growing anaerobes is tedious and time consuming. To grow, anaerobes require a medium prepared for anaerobes and a suitable anaerobic environment. Media for anaerobes needs to be chemically reduced to a level lower than that achieved by removal of oxygen alone. Anaerobes physiologically require this reduced environment to produce energy in order to grow. This level of chemical reduction is achieved by adding chemical reducing agents to the medium. However, the story does not end here. Heat sterilizing a chemically reduced medium with air (oxygen) present leads to the reaction of the reducing agent with oxygen to produce compounds that are harmful to some anaerobes. Once formed, these compounds cannot be removed. To prevent their formation, first oxygen is removed, i.e. make it anaerobic, then the medium is sterilized. This is the way PRAS medium (**P**re-**R**educed-**A**naerobically-**S**terilized) is made.

Commercially produced PRAS medium, like OxyPRAS plates, takes all of the tedium and difficulty out of preparing media for anaerobes. It is specially packaged to maintain reduced and anaerobic conditions and comes ready to use. All of the QC is done.

One of the major suppliers of PRAS media (Oxyrase, Inc.) uses an enzyme biotechnology (The Oxyrase Enzyme System) to remove all oxygen from the medium during preparation. Then the reducing agent is added and the medium is autoclaved. After sterilization, the oxygen-eliminating enzyme is added to the medium again to keep it reduced and anaerobic. The plate is then packaged in special oxygen barrier pouches to maintain the proper oxygen-free and reduced environment.

The resulting PRAS product offers microbiologists a considerably more convenient way to accomplish a difficult job, and provides them more control throughout the entire anaerobe recovery process.

“If we were to go back to using plates that weren't PRAS, then we would have to pre-reduce them before use,” says Mary Stepney, Microbiology Specialist at South Bend Medical Foundation, Inc. (South Bend, IN). “Whenever we have high volumes, and run out of the pre-reduced plates, we could take some out of the refrigerator, but they still could not be used until they are pre-reduced. The ready-to-use OxyPRAS plates keep work moving uninterrupted. Using PRAS also helps to ensure optimal colony development. The growth of anaerobes on OxyPRAS looks healthier than growth on refrigerated commercial anaerobic media pre-reduced prior to use.”

Cassity says one of the reasons why PRAS media grows organisms more quickly is because the medium does not have as much exposure to oxygen or oxidative potential as would often be the case with non-pre-reduced media.

He adds that, while PRAS plates involve a slightly higher out-of-pocket expense than traditional (non-PRAS) plates, the cost difference is often dramatically outweighed by the benefits. For many PRAS media users, the improved quality and reliability of results is noteworthy among those added benefits.

“The quality of the PRAS media is superior, which is very beneficial,” says Stepney. “We’ve never had a product failure or contamination issue, which sometimes occurs with uninoculated plates that are left out and exposed in the processing area. The reliability of the PRAS plate and the individually wrapped packaging makes it very convenient for us.”

To many microbiologists, the superior quality of the PRAS media is enhanced when the plates are thick. This makes possible longer incubation time and increases shelf life.

"The OxyPRAS media is thicker than the standard media," explains Cassity. "It is about six millimeters thick, which is about 50 percent heavier than standard media. That thickness may help prevent tearing of the media on the surface, and makes a difference in the longevity of the plates. That's part of what gives them a longer shelf life. Also, the thickness accounts for a deeper, richer pool of nutrients, which I think adds to the ability to grow bigger, more luxuriant colonies, versus the standard media."

Stepney agrees: "[Due to added media thickness] incubation can be extended without worrying about the plates drying out, which normally happens after four or five days. Since moisture is not lost, the colony size of slow-growing more fastidious anaerobic organisms is not affected."

Netting the cost difference

There is strong evidence that some PRAS media offers clinical microbiologists the advantages of faster and more reliable incubation, larger colonies plus improved shelf life and work time. Additionally, these are also qualities that are beneficial to physicians and patients.

"Growing cultures faster and more luxuriantly is highly beneficial to physicians because it enables them to identify the proper course of treatment more rapidly," says Cassity. "For instance, they can do beta-lactamase testing (or whatever is appropriate) more quickly because the PRAS media saves approximately a day in getting the culture results in the lab. If those results indicate that a therapy change is appropriate, then the physician knows that about a day sooner."

Mary Stepney adds that, due to rising costs, most patients are discharged sooner rather than later. As a result, if it takes too long to get anaerobic culture results reported, the patient may be discharged before the culture results are available - not an ideal situation.

Cassity adds that his lab spends only a few hundred dollars extra per year for PRAS plates, so the added cost is not really an issue. "We watch our expenses very closely," he

says, “but considering our budget is approximately $6 million, a few hundred additional dollars is pretty insignificant – especially if we get results faster and more relevant to the patient’s situation. If you get a patient out of the hospital a day sooner, then you are saving something like $1,400.00 just in hospital costs for that day. We view this technology as a money saver, not an added cost.”

###

For more information, contact Oxyrase Inc*., 3000 Park Avenue West Mansfield, OH; Phone (419) 589-8800; Fax: (419) 589-9919; E-mail:* info@oxyrase.com*; Visit the web site www.oxyrase.com.*