Frequently Asked Questions Regarding 2022 Testing

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Q: Were various devices found to have mycobacteria which were a danger to patients?
A: No. One lab claimed to have found DNA fragments with PCR that fit the "molecular description" of mycobacteria chelonae, and the company followed up to that report with a temporary hold. Following that report, growth studies were performed in four labs, and no positive cultures were found. In addition, various staining techniques were also negative. The company no longer recommends a hold on any of its products and they remain in conformity. No lot or device is in question.

Q: How could a lab claim mycobacterial traces if all the growth studies were negative?
A: Good question. PCR testing protocols usually require positive growth BEFORE testing with PCR methods, because only with positive growth can enough DNA be available to produce a very reliable test. No labs found any positive growth. We don't know how there was enough DNA to use PCR methods without high cycles or other methods that we don't know about.

In cases involving the alleged identification of M. Chelonae (MC), a consultant identified a possible root cause of the misidentification to be improper base-pair complementation of PCR primers with chopped/ segmented nucleic acids resulting from our in-house process of fixing and sterilizing porcine valves and/or very high cycles to "force" a result. That's their opinion.

Other Canadian PCR labs noted that false-positives could have happened, say, due to inadequate positive controls (a separate issue from the lack of DNA available after a negative growth test). In other words, it's very difficult to use PCR on devices with glutaraldehyde in the tissue, given glutaraldehyde is a well-known PCR-inhibitor. So one should compare PCR results from tissue without glutaraldehyde with the devices in question to check if the PCR test is simply impossible to reliably use.

One Finnish lab performed a similar PCR test to identify MC, but cycled their PCR machines to 40-45 to achieve their results. It is our understanding that cycles above 30 consistently lead to false positives. We don't know what the German lab used for cycles, however, as we didn't come across various testing parameters in their reports.

Q: If they are so prone to manipulation, why are PCR machines even used to evaluate medical devices or tissues?
A: From time to time, patients seem to have an infection (fever, halos on CT scans, etc.). Cultures taken from these patients or devices don't always grow, even in perfect lab conditions. This unfortunately happens about 1/3 of the time, so a PCR machine is a "last-ditch" effort to identify an infection so a doctor can prescribe the right antibiotics. In addition, bacteria take time to grow even in perfect conditions, and patients with infections don't have the luxury of several weeks of incubation.

But when cultures of many samples are taken (>30), the chance of a lack of growth (false negative) happening collapses to zero. Despite 50+ samples tested by four labs in four countries, the claim that PCR is the only way to identify the risk of a device to pass on an infection to patients is not credible. Relying on the PCR results alone would require three assumptions: (1) the growth studies were all flawed; (2) that glutaraldehyde and formaldehdye aren't incredibly destructive and adhesive to DNA and other cellular components; (3) there was enough DNA to test without growth first, and (4) that Spontaneous Generation is true--a concept Louis Pasteur proved foolish as we enjoy a century and a half of aseptic tools and techniques. That is, bacteria create bacteria, not destroyed proteins stuck in resin. The growth studies all support this with a unanimously clean result.

Q: If all growth studies with sterilized, packaged products were negative for mycobacteria, how could any agency have issue with the products?
A: The company conjectures that in the wake of the Covid-19 scare, PCR machines have suddenly gained attention among health regulators as a tool for detecting pathogens. However, all sterilized devices, of any kind or construction or manufacturer, have DNA fragments leftover from the sterilization process, so every sterilized device from any manufacturer has DNA that can be "identified" with PCR machines. There is no international or local standard for PCR device use for identifying bacteria, particularly not in devices with highly PCR-interfering chemicals like aldehdyes, and particularly not without a positive growth study. The only standard is growth incubation studies. Again, no Canadian PCR labs believed the tests were done correctly especially without growth.

Q: So if the devices were all sterile, why did BIS leave the market?
A: Once there was no positive cultures and the feedback from PCR labs in Canada was that PCR data were moot, the company moved to lift the hold globally. However, the Europeans rejected the idea and wanted to continue its investigations. It was at this point that BIS realized the EEA weren't serious about moving forward and allowing these "last resort" devices to be used, but rather keeping restrictions and product worries in play indefinitely. BIS left the EEA market in protest, and lifted the hold globally.

Q: So is there a chance a patient could have been infected after implant of the product?
A: The valves tested are all indicated ONLY for patients with infections, where no other options were available. If a patient received a BioIntegral Surgical, Inc. valve implanted, it was because they had an existing infection or was at a very high risk of infection. Unfortunately, all implanted patients had infection risks unrelated to the devices. The average rate of infection from valve use was in line with the homograft, considered the gold standard for infection resistance (and the device to use BEFORE contemplating use of the BioIntegral Surgical products).

Q: Scares happen. Did the company review any of its systems, such as sterilization just to be sure?
A: Yes. The company uses the greatest number of different sterilants versus heart valve manufacturers. These include various aldehydes and alcohols. According to 3rd party studies, after hunderds of hours of exposure to these chemicals, the devices have a one in 10^{32} chance of having any viable bacteria. That's far beyond an SAL of 6, which is the industry standard. BIS employs an "overkill" method for all sterilization processes, vastly increasing confidence intervals as safety.

In addition, the sterility validation and verification data were reviewed, and there were no deviations. The

sterility packaging validation is the most sensitive and extensive in the industry. No other heart valve company uses such a robust system to ensure all sterilization systems are functioning properly.

Q: Perhaps there was a mycobacterial infection in the facility which the German lab picked up?
A: Mycobacteria were never identified in the facility or the devices. In addition, the all the solutions which touched the devices were either (1)bacteriocidal with high exposure times or (2)were sterile fuilds purchased by third parties, suitable for use for direct human injection. There is no internal data to suggest an issue and no credible source of mycobacteria.

Q: Could any mycobacteria have come from the slaughterhouses from which the porcine valves were harvested?
A: As we all know, pigs naturally carry many diseases, bacterial and viral. But mycobacteria chelonae is not a bacteria found in pigs. As importantly, fresh, raw, untreated valves straight from the slaughterhouse were tested for mycobacteria in culture and they were negative.

Q: Why was there concern about the devices in government bulletins?
A: Good question. The reporting of infections with the devices is in-line with historical numbers. Vigilance reports indicate that doctors still continue using the devices, so it's only the agencies that seem preoccupied with the idea. Physicians use the devices as a last line of defense for infection after other options have failed.

Q: Wasn't there a large rise in infections from these devices?
A: No. The vigilance profile for infection has not changed over the past few decades of data collection. Although curiously, there has been a spike of infection reports from three centers in Germany (notably, they were all patients with high risk for infection, as per the IFU). However, these centers have used the devices for infections for nearly a decade and in one reported case, the surgeon used a BioIntegral device in the patient after explanting the first one. In other words, other countries have not indicated a problem other than the country that initially pushed the PCR study results.

Q: Did any DNA cause clinical issues?
A: All xenografts and human homografts have DNA, and there is no evidence in the literature that they lead to clinical issues. It is typical to find DNA fragments in all biological tissue, from all sources, in all types of collagen-based devices, regardless of tissue type, source or final sterile device configuration. More broadly, all fixed xenografts contain cell particles, fixed into the tissue's collagen walls, and have been part of every implant in the industry since the 1950s. What's more, the gold standard of biological heart valve replacement, the human cadaver homograft, contains whole human DNA given they are not processed prior to implantation. They are the gold standard of infection resistance and the Instructions for Use for the valves indicate they should be used first before using BioIntegral devices. Lastly, glutaralahyde fixation both denatures the DNA strands as well as mummifies cellular components into the collagen tissue. It is unlikely that they can become sessile or freely available after implantation.

In addition, all device lots are tested for the possibility that proteins could case a reaction to patients. All device lots passed these tests, or else they wouldn't have been released.

We reiterate, the test results that formed the basis for the initial causes for concern were invalidated. Growth studies performed over several months of incubation have found no growth in any of the cultures, in any of the labs, including the labs which originally claimed to identify organisms with PCR only. It's a requirement of PCR studies to have growth first, and then identification second. **It's clear that no product had viable microorganisms, of any kind. The sterility processes used at BioIntegral Surgical have been well-validated to deactivate mycobacteria and more chemically-resistant organisms, and years of test results support their effectiveness. According to test data, the chances of having contracted any mycobacteria from a BioIntegral Surgical device is close to zero.**

Q: What causes mycobacterial infections?
A: All mycobacteria claims were driven by the major predictive variables: multiple operations and environmental factors. Put more simply, they tend to come from contaminated water and human skin. Many patients who have had cardiac surgery also have multiple catherterizations, which are inserted into the body via skin punctures.

An expanded PMS was initiated to look for possible mycobacteria cases. However, the only patterns uncovered for mycobacteria cases confirmed with growth were multiple operations (the major factor) and environmental factors (skin contamination, hospital water system anomalies). It is well known that mycobacteria are widespread through the environment, and given the large amount of test data to date, it is not reasonable to assume that any MC infections would have come from the BioIntegral Surgical devices given the potent sterility agents used and sterility validations and verifications.

Q: Did surgeons use the devices correctly to minimize infections?
A: A recent PMS review did indicate that many surgeons DO NOT use anti-coagulation for post-implant care, as indicated in the *Instructions for Use* for complication/infection minimization. The rationale for anti-coagulation is to improve healing, avoid fibrin build-up (a major site for bacterial infections), and eventually reduce/avoid common problems such as infection, distal stenosis, and other issues. Some surgeons recently have been concerned the presence of DNA might have affected clinical outcomes, but there is no evidence that that is the case, in any xenograft implant from any manufacturer. The lack of use of anti-coagulants to improve infection protection or obviate other fibrin-related complications like distal stenosis is regrettable, and we urged surgeons to follow the instructions and recommendations in the IFU.