Field Safety Notice

(1) No-React® BioConduit (NRAC),
(2) No-React® BioPulmonic Conduit (NRPC),
(3) No-React® Injectable BioPulmonic (NRIP),
(4) No-React® BioMitral (NRM),
(5) No-React® BioAortic (NRA)
FSCA-003-22

Date: 2022-08-03

Attention: Distributors of BioIntegral Surgical Heart Valves outside of the EEA/UK **only**

Devices: bioprosthetic heart valves (1) No-React® BioConduit (NRAC), (2) No-React® BioPulmonic Conduit (NRPC), (3) No-React® Injectable BioPulmonic (NRIP), (4) No-React® BioMitral (NRM), (5) No-React® BioAortic (NRA)

Update: *All holds or restrictions related to mycobacterial investigation were LIFTED July 2022. Below is a summary of the investigation results for customers who asked for more information.*

Executive Summary: No mycobacteria were found in any devices (no growth, no positive stains, no PCR results), over months of manufacturer testing in several labs in the EU and Canada. By all investigative routes to date, BioIntegral Surgical devices have not deviated from standards of conformity with regards to biocompatibility, sterility, pyrogenicity, contamination or storage. Despite concerns raised by some DNA research labs in Europe, BioIntegral's laboratory and all field data suggest that the devices remained sterile and safe at the levels assured by the QMS and in compliance with state of the art, harmonised standards. *The hold from April 2022 might have been a premature reaction to PCR analysis performed without proper, positive controls for fixed tissue.*

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. **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.**

No mycobacteria (or any bacteria) found using any test method over the past several months. While concerns were raised by some in early 2022 that viable *Mycobacteria chelonae* could be present in devices, the multi-laboratory EU/Canadian investigation to date has not shown any positive growth in culture on dozens of sterilized devices tested, of any type of organism. Canadian labs have yet not been able to duplicate positive staining (negative) and PCR test results, complicated by the fact that no growth was observed (i.e., inadequate DNA to test, PCR not a usable test for fixed tissue). As of the date of this bulletin, our experts surmise that it's possible the proper positive controls were not used in the original testing (e.g., inadequate recovery, well-known glutaraldehyde interference), which led to the original (false) alarm.

Part of the investigation included confirming previous PCR test results, which were invalidated by Canadian labs and local disease specialists. Throughout all the investigations since April 2022, no EU lab has been able to specify what positive/negative controls were used for the original PCR results. Canadian labs couldn't begin to test the devices for DNA of any organisms, given no proper controls and no prior growth in special media. While we are still investigating, all labs we have been working with suggested that the original results might have been the result of PCR parameters that would normally yield false positives. What's more, the kits used for the original testing in the EU were recalled in Canada due to contaminations and organic debris. This is yet all the more reason that proper controls were necessary and vital *before manufacturers put holds on their products*.

PCR labs and various experts have enumerated some of the issues with the PCR testing that formed the basis of the lifted hold:

- Slicing/cutting/homogenizing the valves exposes preserved bacterial DNA/RNA from beneath tissue leading to false positives from non-viable porcine mycobacteria that originated from living animal that have been fixed/ preserved within the tissue
- An EU lab initially stated they had 'not ideal' cultures of mycobacteria from an explanted valve but still ran an unknown/generalized PCR process. No labs have been unable to culture viable/live mycobacteria from any BIS valves
- No EU labs have shared their PCR validation data with BioIntegral Surgical (i.e. CT cycle threshold cut-offs, primer sequences, extracted DNA/RNA integrity etc.)
- No EU lab that reported *M. Chelone* had asked us to produce a glutaraldehyde-fixed + mycobacteria-injected positive control for them. So, no valid/accurate positive controls have been made using our unique manufacturing processes
- Nucleic acids are stressed/damaged with our glutaraldehyde process, which will damages any possible bacteria DNA. FISH staining detects nucleic acids in the spatial cellular environment. Chopped DNA/RNA from the glutaraldehyde process may give FISH-false positives. The EU labs did NOT ask us about our glutaraldehyde/formaldehyde process before their FISH staining and PCR
- Multiple labs had no mycobacteria growth for several months, which were the best/ideal conditions for mycobacteria growth. The human body is a far less ideal condition for growth. It is reasonable to suggest that in patients with infections YEARS after a BIS valve implantation likely got infected from a more recent source than the earlier BIS valve implant
- To date, no EU lab has shared the primer sequences that were used to detect the M. Chelone bacteria identified in the BIS valves
- The harshness of our glutaraldehyde/formaldehyde sterilization processes is so severe--and for such a long duration--that no organism could possibly have survived. There was ample literature to show this, as well as years of internal validations/verifications. In addition, any DNA/RNA is also very thoroughly destroyed/fragmented in this process and is not accounted for by original labs that did testing
- Glutaraldehyde stresses/chops nucleic acids within bacteria (i.e. RNA/DNA). FISH staining and PCR depend on nucleic acid integrity for accuracy. ***PCR labs in Canada did not believe there was enough integrity after processing to yield accurate results to accurately identify any organism in particular and refused to perform testing on the fixed tissue***

- At no point did the original labs approach BIS to inquire about the company's glutaraldehyde and formaldehyde processes. This would have had critical information regarding DNA/RNA segmentation/integrity that is important for successful FISH staining. At best they could have come to BIS and asked us to produce a positive control with them, which they did not
- No EU lab has approached BIS to produce any positive controls (using our glutaraldehyde process) to be used in the PCR testing to ensure accurate detection of M. Chelonae
- Ever since the lab's 'not ideal' culture of mycobacteria, there has been no growth from any of the cultures from our valves. Even though cultures were grown for as long as 20 weeks
- All mycobacteria studies stress the need for culture confirmation prior to a positive identification of the type of mycobacteria. None of the original labs' results have been reproducible besides the PCR, which was done using the Hain Lifescience kits (which have a history of recalls for contamination). To date, primer sequences for any confirmation PCR testing has not been shared by any 3rd party laboratory

In cases involving the alleged identification of M. Chelonae, the root cause of the misidentification was likely due to improper base-pair complementation of PCR primers with chopped/segmented nucleic acids resulting from our in-house process of fixing and sterilizing porcine valves. Unfortunately, it seems that the initial lab PCR results were not done on cultured non-implanted mycobacteria as is necessary. The labs that originally tested our products likely reported false-positives due to inadequate positive controls. These labs could not have used adequate positive controls as none had approached us to reproduce fixation and sterilization processes (which segments DNA within the valves and confounds results). Crucially, none of the initial (or subsequent) labs that reported staining and PCR were able to grow viable mycobacteria from non-implanted BIS valves.

Sterility Risk Analysis indicates BioIntegral Surgical uses the widest spectrum of sterilants in the industry, making microbiological resistance a non-issue. In early conversations with regulators, concern was raised regarding glutaraldehyde-resistant strains could be present in the devices. However, BioIntegral Surgical is the only heart-valve manufacturer that uses three sterilizing agents, formaldehyde being the most potent. Other heart valve companies only employ glutaraldehdye, the agent most likely to afford resistance. In addition to a variety of virucides/bactericides, the company employs an "overkill" method of long exposures (hundreds of hours of aldehyde exposure).

A review of decades of manufacturing data and various sterility tests confirm the devices did not have viable organisms. BioIntegral's QMS requires periodic incubation of sterile devices as a very detailed check of the sterility processes. This included thousands of samples over many years, many of which were incubated for months, not just the lab-normal 14 days. This is outside of 3rd party sterility and LAL test results for each lot, whose passing is a requirement for release of inventory.

A review of post-market surveillance did not indicate any anomalies with respect to expected adverse events. For example, NRAC BioConduit continues to have adverse event rates lower than its gold-standard comparison, the human cadaver homograft. This is in the context of its indication for use only if a homograft is unavailable. The NRPC's major adverse event continues to be distal stenosis, similar to all pulmonary conduits, regardless of human or xenograft/manufacturing origin.

All mycobacteria claims were driven by the major predictive variables: multiple operations and environmental factors. An expanded PMS was initiated to look for possible mycobacteria cases. However, the only patterns uncovered for mycobacteria casese 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.

A recent PMS review did indicate that some surgeons did not use anti-coagulation for post-implant care, as indicated in the *Instructions for Use* for complication minimization. The rationale for anti-coagulation is to improve healing, avoid fibrin build-up, 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. The lack of use of anti-coagulants to improve infection protection or obviate other fibrin-related complications like distal stenosis is regrettable, and we urge surgeons to follow the instructions and recommendations in the IFU.

All xenografts and 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. 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 after implantation.

* * *

By relevant investigative routes carried out to date**, BioIntegral Surgical devices do not seem to have deviated from Standards of Conformity with regards to biocompatibility, sterility, pyrogenicity or storage**. There is almost always DNA in harvested, sterile animal tissue. Concerns about DNA in device tissues must be put into the context as all sterile, fixed tissues harvested from live animals are likely to include the presence of a variety of DNA fragments, regardless of source or manufacturer.

Advise on action to be taken by the user: no hold / restrictions on any products outside the EEA/UK.

Transmission of this Field Safety Notice: This notice needs to be passed on all those who need to be aware within your organization (distributors) or to any organization where the potentially affected devices have been transferred outside of the EEA/UK.

Please transfer this notice to other organizations (e.g., distributors' customers) on which this action has an impact outside of the EEA/UK.

S. Gabbay, MD
Medical Director and CEO