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LyoDots^{TM} (lyophilized reagents in a flat, circular shape): A Unique Lyophilization Format for Next Generation Point of Care Testing

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Abstract

Argonaut Manufacturing Services (Argonaut) has demonstrated a new configuration of lyophilized material where the lyophilized reagent is in the form of a flat, circular, dome shape called a LyoDot^{TM}. This shape allows the lyophilized material to readily dissolve and accommodate small spaces, such as a shallow-depth chamber in a diagnostic device. To create a LyoDot, a liquid reagent is applied to a proprietary treated film surface and subsequently lyophilized. The film provides mechanical support, and the underside of the film allows for adhesive application, which may be used to attach the LyoDots to specific locations. LyoDots can be easily integrated into device assembly using standard pick and place automation due to their robust nature and planar X-Y coordinate layout. As a proof of principle, LyoDots were created using RT-qPCR assay reagents from Fortis Life Sciences and multiplexed oligonucleotides corresponding to the CDC 2019 nCoV RT PCR assay. The LyoDots had a diameter of 3.75 mm and a thickness of 390 µm. When the LyoDots were functionally tested, they were found to produce results that were comparable to liquid reagents prior to lyophilization. To highlight utilization of LyoDots in diagnostic device applications, two LyoDots, containing dye, were placed in a chamber that was 0.5 x 0.5 x 0.030 inches. The LyoDots were placed adjacent to each other and were attached to the surface via adhesive which was applied to the underside of a clear film. Upon exposure to liquid, the LyoDots readily reconstituted, and their contents remained on their respective side of the chamber. The LyoDots exhibited attributes of fast dissolution and ability to maintain spatial control of reconstituted lyophilized material making them attractive for use in microfluidic devices or small point of care tests, which are growing rapidly in the molecular diagnostic market. This communication, demonstrating LyoDot functionality and implementation in a microfluidic device, shows the potential applicability of LyoDots toward many types of devices. LyoDots enable high volume, cost effective, “automation friendly” formats required for next generation point of care testing.

Introduction

The COVID-19 pandemic highlighted the need for rapid and sensitive diagnostic tests which are stable at room temperature and are easily transported. One way to achieve this is through the incorporation of lyophilized reagents. Argonaut makes a variety of lyophilization services available to life science companies. For example, the LyoDose^{TM} bead technology, which consists of a sphere with lyophilized chemistries in amounts needed for a single reaction (Figure 1), has been utilized by many companies for years to enhance their products.

Figure 1. LyoDose bead, a sphere of lyophilized reagents that can be used to support a single reaction in a device or well, shown in a tube. These applications of LyoDose beads have been successful for years. The incorporation of lyophilized material has many benefits including elimination of cold chain storage, flexibility in assembly, increased stability, and assay simplification.

Although the spherical shape of a LyoDose bead is compatible with many devices, the need to reduce device footprint requires a thinner lyophilized material geometry. In this communication, we report on a new configuration of lyophilized material where the lyophilized reagent is in the form of a flat, circular, dome shape called a LyoDot (Figure 2).

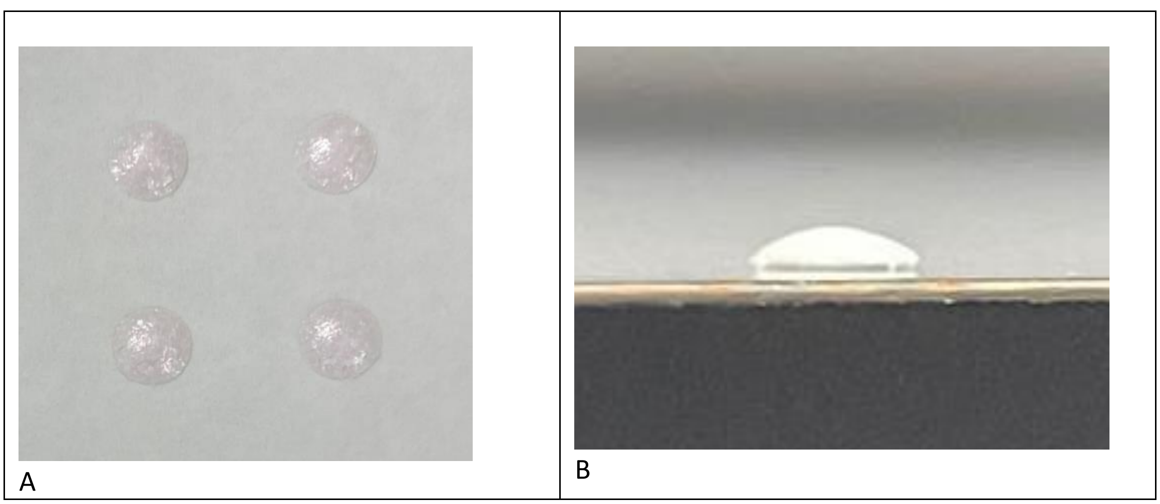


Figure 2. Top view image of LyoDots (A) with a diameter of 3.75 mm. Side view image of a LyoDot (B) with a thickness of 390 µm. The LyoDots consisted of reagents needed for the CDC 2019-nCoV RT-PCR assay, which were provided by Fortis Life Sciences.

The shape of the LyoDot allows a similar amount of lyophilized material to fit into thinner spaces compared to LyoDose beads containing the same amount of lyophilized material. For example, a LyoDot made with volume of 5 µL of liquid reagent (before lyophilization) was more than 5X thinner than a LyoDose bead made with the same volume of liquid reagent. To create a LyoDot, a liquid reagent is applied to a proprietary treated film surface and subsequently lyophilized. The film provides mechanical support, and the underside of the film allows for adhesive application, which may be used to attach the LyoDots to specific locations. LyoDots can be easily integrated into device assembly using standard pick and place automation due to its robust nature and planar X-Y coordinate layout. These attributes of LyoDots further enhance device miniaturization while potentially lowering reagent cost.

LyoDot Technology

To demonstrate the technology, Argonaut partnered with Fortis Life Sciences (Empirical Bioscience, Inc.), a developer and manufacturer of enzymes, proteins, and reagents for molecular biology, to create LyoDots using Lyo-Ready RT-qPCR master mix combined with multiplexed oligonucleotides corresponding to the CDC 2019-nCoV RT-PCR assay (N1 assay = FAM, N2 assay = HEX, RNase P assay = ROX). The LyoDots had a diameter of 3.75 mm and a thickness of 390 µm (Figure 2). Synthetic SARS-CoV-2 RNA template (Twist Bioscience) was added to the RT-qPCR reactions (20 uL final volume per reaction) at 5×10^5, 5×10^4, 5×10^3, 5×10^2, 5×10^1 or 0 (negative control) copies. To simulate a clinical sample, Universal Human Reference RNA remained constant at 1 ng per for each condition. LyoDots were resuspended in molecular grade water at 15 µL of water per one LyoDot, and the resulting suspension was distributed to qPCR plates at 15 µL volumes per well. Then, template (5uL) was added to the wells, followed by RT-qPCR. Non-lyophilized liquid reagents were also tested. Samples were tested in triplicate for each condition.

The results for the RT-qPCR testing are included in Figures 3 and 4. The resuspended LyoDot material demonstrated similar dynamic range and sensitivity when compared to the non-lyophilized liquid reagents. Negative controls (no nCoV template and with Universal Human Reference RNA) demonstrated amplification for RNase P while N1 and N2 had no detectable amplification. CDC 2019-nCoV RT-PCR N1 and N2 assays displayed ~100% PCR reaction efficiency for both sets of reagents, with nearly identical Ct values. The CDC 2019-nCoV RT-PCR RNase P assay Ct values remained similar throughout the experiment, except when the copy number of nCoV synthetic RNA was at 500,000 copies per reaction, in which case RNase P was 2 Ct higher. This higher Ct was found in both non-lyophilized liquid reagents and LyoDots. The higher Ct is likely due to reagent component (e.g., oligonucleotides, dNTPs), depletion amplifying the high copy RNA, in this case the N1 and N2 targets, leaving less efficient amplification of the lower copy RNase P target. Overall, the results demonstrate equivalent performance of the non-lyophilized liquid reagents and LyoDots.

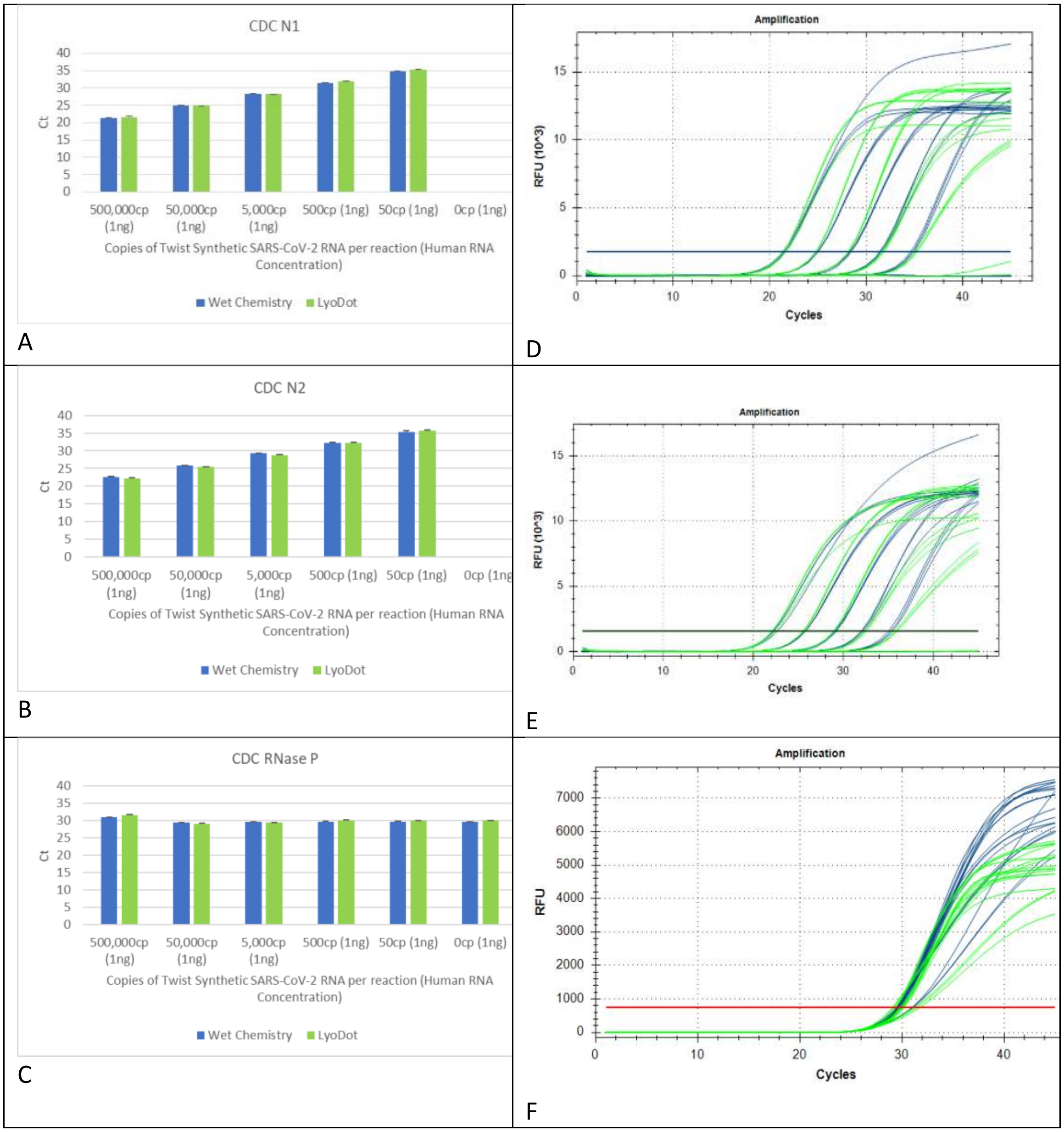
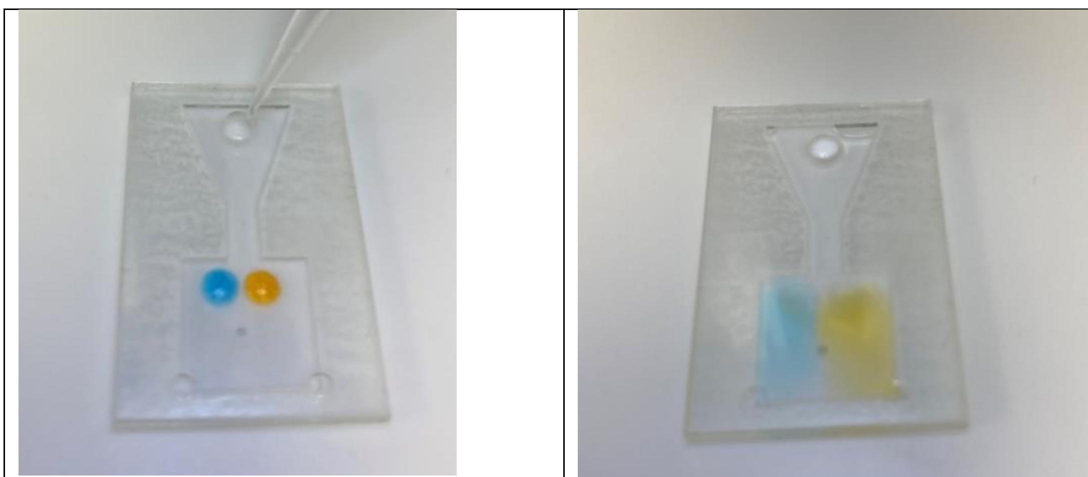


Figure 3. RT-qPCR results for the CDC 2019-nCoV RT-PCR assay using LyoDot material (green) compared to the non-lyophilized liquid reagents (blue). In this experiment, synthetic SARS-CoV-2 RNA was varied, while the concentration of RNase P template was held constant at 1ng per reaction. Each sample was run in triplicate. Bar charts of the Ct values for the CDC 2019-nCoV RT-PCR N1 (A) and CDC 2019-nCoV RT-PCR N2 (B) assays at different copy numbers are shown. A bar chart of the Ct values for the RNase P template (C) at a constant copy number with variable amounts of SARS-CoV-2 RNA is presented. The corresponding amplification curves for the CDC 2019-nCoV RT-PCR N1 (D), CDC 2019-nCoV RT-PCR (E), and CDC 2019-nCoV RT-PCR RNase P (F) assays are also shown.

			Copies of Twist Synthetic SARS-CoV-2 RNA per Reaction (Human RNA Concentration, ng per reaction)								
			500,000cp (1ng)	50,000cp (1ng)	5,000cp (1ng)	500cp (1ng)	50cp (1ng)	0cp (1ng)	Slope	Efficiency	R2
Wet Chemistry	CDC N1	Mean Ct	21.38	24.91	28.26	31.39	34.68	N/A	-3.308	100.60%	0.999
		Stdev	0.107	0.019	0.065	0.09	0.168				
	CDC N2	Mean Ct	22.53	25.91	29.26	32.24	35.38	N/A	-3.203	105.20%	0.998
		Stdev	0.264	0.027	0.08	0.142	0.215				
	CDC RnaseP	Mean Ct	31.05	29.41	29.54	29.66	29.63	29.62	N/A	N/A	N/A
		Stdev	0.017	0.028	0.059	0.141	0.116	0.031			
LyoDot™	CDC N1	Mean Ct	21.6	24.89	28.26	31.82	35.28	N/A	-3.429	95.70%	0.999
		Stdev	0.195	0.031	0.022	0.137	0.092				
	CDC N2	Mean Ct	22.11	25.38	28.82	32.25	35.69	N/A	-3.405	96.60%	1
		Stdev	0.179	0.035	0.044	0.149	0.112				
	CDC RnaseP	Mean Ct	31.47	29.14	29.39	30.11	30.05	29.92	N/A	N/A	N/A
		Stdev	0.292	0.035	0.007	0.086	0.023	0.059			

Figure 4. Tabular data of the RT-qPCR results corresponding to the results from Figure 3 for the CDC 2019-nCoV RT-PCR assay for LyoDot material compared to the non-lyophilized liquid reagents. Each condition was run in triplicate and contained SARS-CoV-2 RNA and RNase P template as indicated.

To illustrate the use of LyoDots in a device, LyoDots containing dye were incorporated into an acrylic microfluidic chamber with dimensions of 0.5 x 0.5 x 0.030 inches (Figure 5, A). LyoDots were adhered to the surface of the chamber by placing adhesive to the underside of a clear, plastic layer connected to the LyoDot to prevent movement. After water was introduced to the device, the LyoDot components readily dissolved and remained on their respective side of the chamber (Figure 5, B), demonstrating spatial control of the dissolved lyophilized material. This proof-of-principle demonstrates that LyoDots could be used in devices with shallow chambers where efficient storage, dissolution, and transfer of reagents are needed. Applications may include immunoassay and molecular assays.



A B

Figure 5. Device containing colored LyoDots in a chamber before addition of liquid (A), and the same device after the addition of water to the device (B). The LyoDots were attached to the chamber, which was made of acrylic and had dimensions of 0.5 x 0.5 x 0.030 inches. When exposed to liquid, the LyoDots readily dissolved and remained in their respective side of the chamber.

A key manufacturing factor is robustness in handling. We demonstrate LyoDots were compatible with handling using air tweezers (Figure 6, A). For adaptability with high-throughput manufacturing methods, the LyoDots can also be configured into sheets (Figure 6, B and Figure 7).

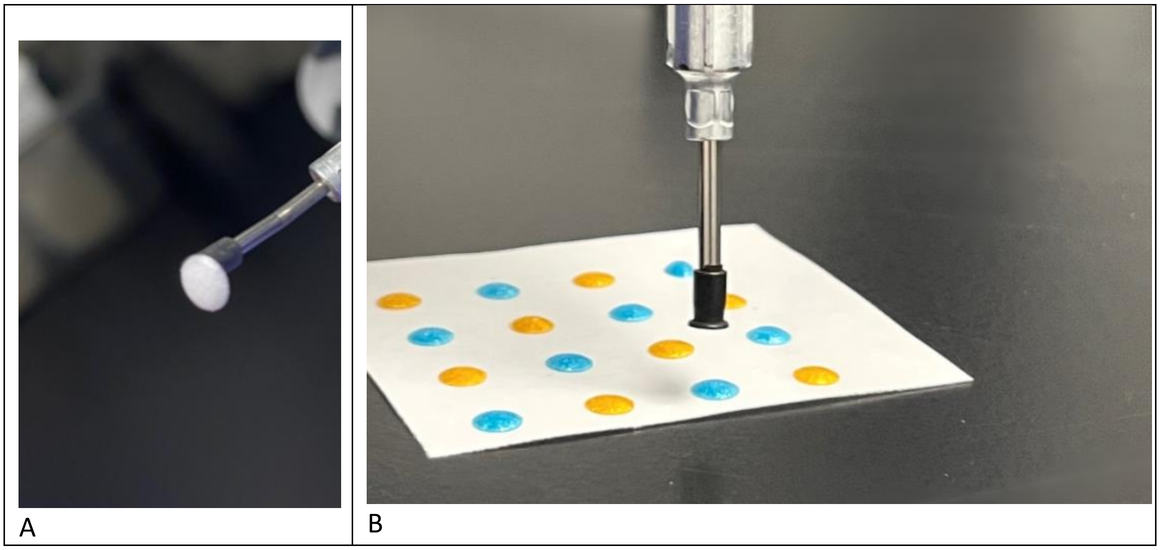


Figure 6. LyoDot being handled by air tweezers (A) and LyoDots presented on a sheet to air tweezers (B).

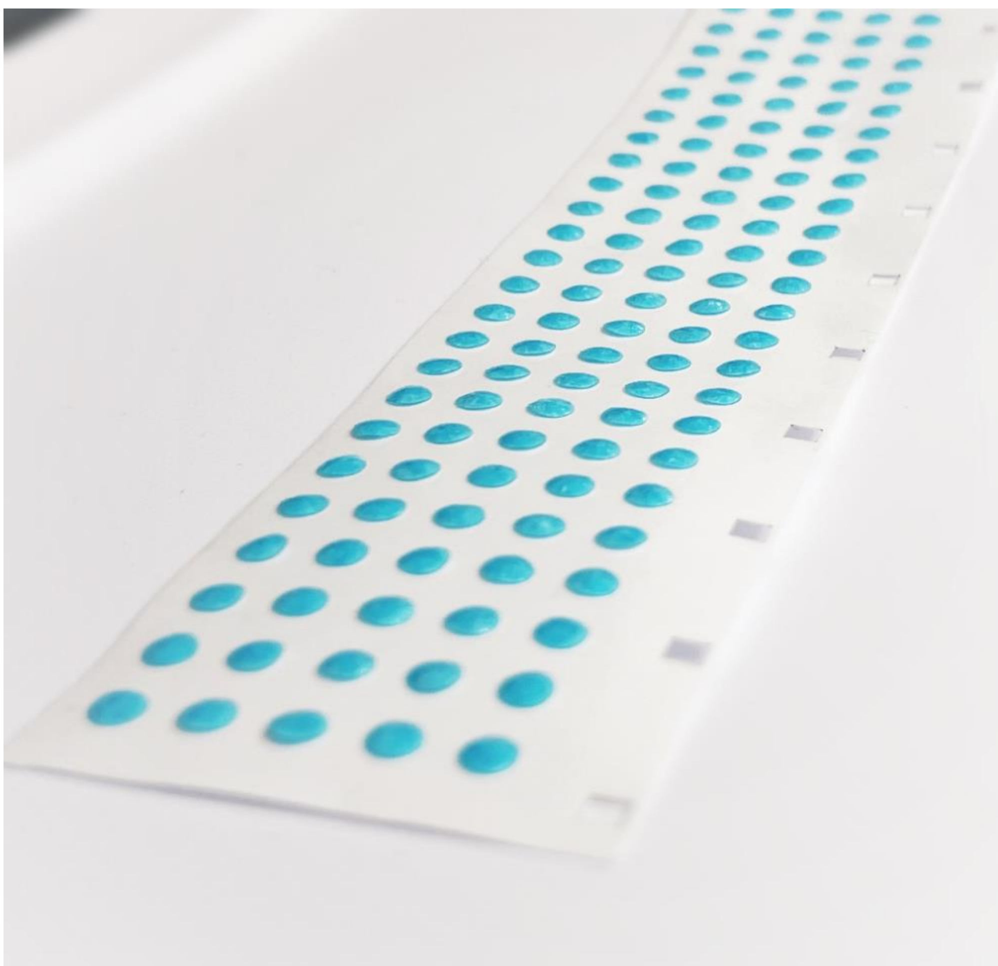


Figure 7. LyoDots configured in strips illustrating potential compatibility with manufacturing automation.

Finally, LyoDots can offer a dramatically lower landed cost advantage in high volume molecular applications. Compared to liquid reagent volumes, applications using LyoDots can be reduced for the first time below the sub-2µL level, while still maintaining a manageable working size for a manufacturing setting. Lower volumes are in development.

This communication presents a novel configuration of lyophilized material called a LyoDot, which is a flat, circular, dome shaped lyophilized reagent attached to a support film. The LyoDot format is suitable for the constrained spaces required in device miniaturization with numerous applications including point of care testing. The LyoDots readily reconstitute upon exposure to liquid and can be adhered to surfaces by attaching them to a proprietary clear, plastic film. In this communication, we demonstrate functional LyoDots can be made using reagents in a CDC 2019-nCoV RT-PCR assay and these LyoDots had similar activity compared to the corresponding liquid, non-lyophilized reagents. LyoDots were also incorporated into a spatially constrained environment and demonstrated rapid reconstitution with spatial control of the dissolved contents. This new configuration of lyophilized material has the potential to be compatible with shipping, handling, and manufacturing methods in high volume automation production environments. The ability to introduce scalable LyoDots provides unique lyophilization services that further enables next generation point of care devices.

Patents

LyoDots may be manufactured under or covered by one or more pending U.S. patents owned by Argonaut Manufacturing Services. This serves as notice under 35 U.S.C. § 287(a) for the LyoDots product.