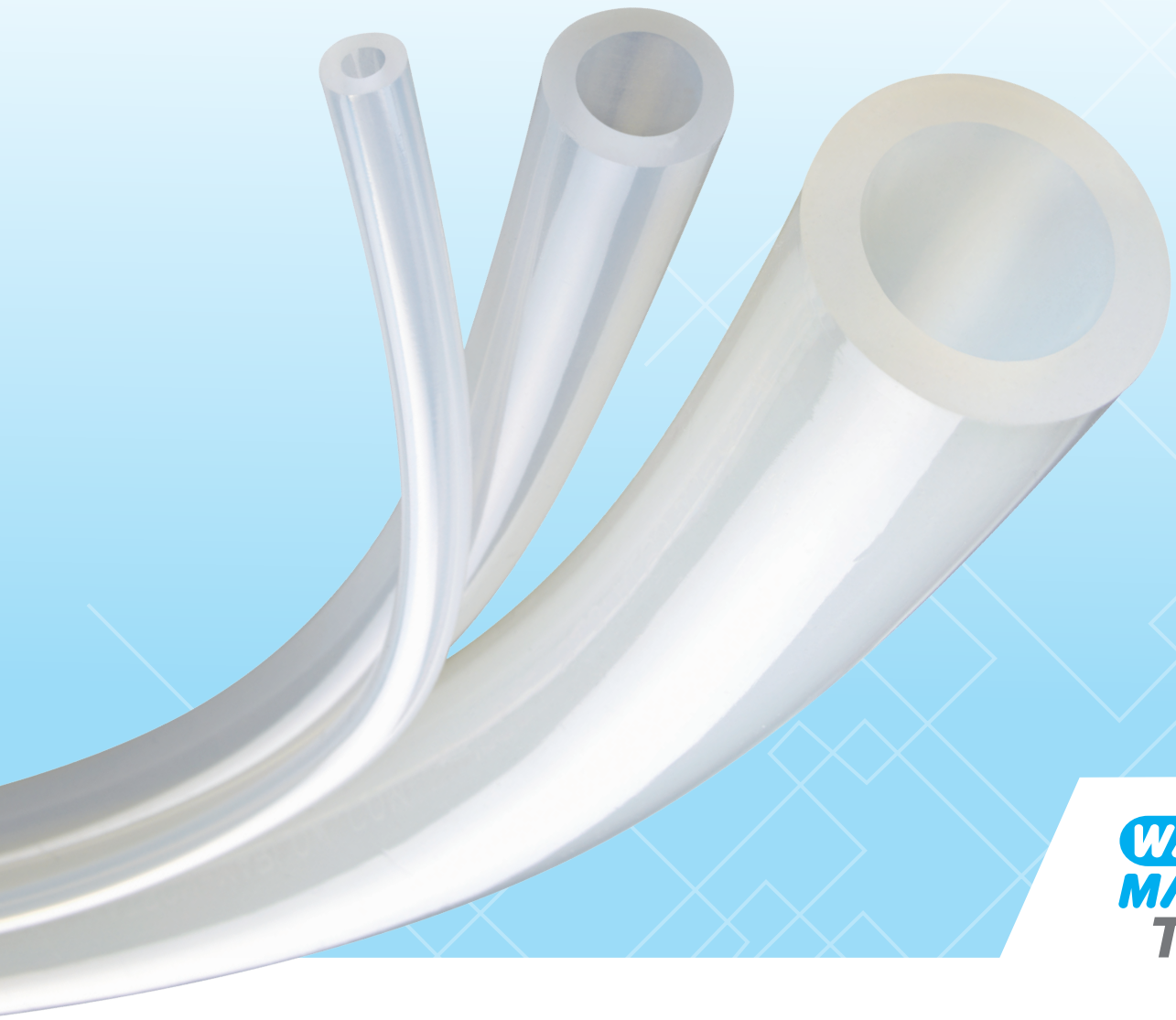
WATSON MARLOW Fluid Technology Solutions



WATSON MARLOW Tubing

FULL VALIDATION GUIDE

Pumpsil® Issue 12

Executive Summary

This is the full validation guide for Pumpsil tubing. It contains additional hyperlinks to the test reports. The additional hyperlinks are provided in section 8 page 10. This should provide the reader with detailed test procedures and actual test results.

CONTENTS

1. Introduction

Watson-Marlow's Pumpsil 913.A and 913.B series platinum-cured silicone tubing is a high purity, 'addition cured' silicone which is USP Class VI and ISO 10993 compliant, and produced from a USP Class VI raw material. It is manufactured in an ISO 14644-1 class 7 cleanroom. The cleanroom is temperature and humidity controlled, thereby providing a stable extrusion environment.

Pumpsil 913.A and 913.B series platinum-cured silicone tubing exhibits a number of key features:

- **Ultra-smooth bore to control protein binding and bacterial growth. Tight surface cure. Non-tacky surface**
- **Traceable with laser etching of the part number, lot number and use-by date along the length of the tube. Full traceability is therefore assured even when the tubing is removed from the bag**
- **Excellent flow stability for accurate process control**
- **Very low extractables, non-toxic and Animal Derived Content Free (ADCF)**
- **Comprehensive stock of a wide range of sizes**

Pumpsil is fully post-cured, a process which removes cyclic siloxanes and other volatiles, resulting in an exceptionally pure tube. Another major benefit of post-curing is increased cross-linking between molecules. These tighter molecular bonds maximise resistance to flexing stress. The tubing fatigues more slowly and therefore retains its original shape for longer and dispensed volumes remain accurate throughout the tube life.

Watson-Marlow Fluid Technology Solutions (WMFTS) is the world leader in niche peristaltic and sinusoidal pumps and associated fluid path technologies. Founded on nearly 60 years of supplying engineering and process expertise and with over one million pumps installed worldwide, our pumps are tried, tested and proven to deliver.

WMFTS is a wholly owned subsidiary of Spirax-Sarco Engineering plc (LSE: SPX), a global organisation employing approximately 4,800 people worldwide. Watson-Marlow Fluid Technology Solutions comprises nine established brands, each with their own area of expertise, but together offering our customers an unrivalled breadth of solutions for their pumping applications.

2. Irradiation and autoclave conditions and working temperature

Pumpsil tubing may be sterilised using either of the following methods:

- **Gamma irradiated to 50kGy**
- **Autoclaved at 121C for up to 60 minutes**

Working temperature range

- **-20C to +80C**

The working temperature range for Pumpsil 913.A and 913.B series silicone tubing has been determined based on its use as a peristaltic tube in Watson-Marlow pumps. For static (i.e. non-pumped) applications these limits may be exceeded, but it is recommended that testing is carried out to ensure suitability.

3. Chemical compatibility

A general guide on chemical compatibility of Pumpsil tubing can be found on Watson-Marlow Fluid Technologies Solutions website. *wmfts.com/chemical*

4. Regulation compliance statements and manufacturing conditions

4a Materials of construction

Pumpsil is made of polydimethylsiloxane (PDMS) structure with a high surface area silica filler to provide enhanced physical characteristics. The tube contains less than 1% catalyst residue, which itself is primarily a silicone carrier for the platinum catalyst. Platinum is not detected in the aqueous extracts from the finished tube (see section 6).

4b Manufacturing environment

Pumpsil is manufactured according to current Good Manufacturing Practices (cGMP) in an ISO 14644-1 Class 7 cleanroom.

4c Country of origin

Pumpsil is manufactured in Falmouth, Cornwall, United Kingdom.

4d Compliance declaration summary

Table 1 details the different substances that are not present in the raw material, manufacturing process or final composition of Pumpsil.

For full compliance statements please refer to the compliance summary sheet available from the website or WMFTS representative.

4e REACH legislation

All raw materials, compounds used in the manufacturing process and the final Accusil product comply with the REACH regulation. None of the chemicals used in the manufacture of Accusil are on the candidate list of substances of 2008 or the list of Substance of Very High Concern (SVHC).

4f RoHS

In compliance with the restriction of hazardous substances (RoHS) directives, no listed substances are used in the manufacture of Accusil.

4g Shelf life storage conditions

The use-by date of the tubing is included on the bag and box label. To maintain the performance of the tubing throughout its life, tubing should be stored in a cool, dry environment away from direct sunlight. The optimum storage temperature is between 18-21C (65-70F). However, normal warehouse conditions of 5-30C (40-86F) are acceptable. Wherever possible, original packaging should be maintained. Stock should be rotated on a first in, first out (FIFO) basis.

The performance of any tubing beyond its use-by date, or which has not been stored according to the recommendations outlined above, cannot be assured.

Table 1: List of compliance statements for Pumpsil and substances not present in the manufacture of Pumpsil

Named substance/compliance statement	Raw material	Manufacturing process	Final product
Gluten	-	-	-
Animal Derived Content Free (ADCF)	-	-	-
Melamine	-	-	-
Phthalates	-	-	-
Bisphenol A (BPA)	-	-	-
Latex	-	-	-
Allergens (as defined by FDA CFR 21.164.110)	-	-	-
Presence of heavy metals	-	-	-

'-' denotes not present or not added

5. Biocompatibility and physiochemical testing

5a Summary table

Table 2 contains a summary of all the compendial testing and ISO qualifications that Pumpsil has been evaluated for. Full test methods and results are available on request.

Pumpsil has passed a number of compendial and ISO testing, a summary of the results are disclosed within.

Table 2: A summary of the compendial and ISO tests performed on Pumpsil

Test reference	Test description	Result
ISO 10993-11	Systemic toxicity	PASS
ISO 10993-10	Intracutaneous injection – test for irritation and skin sensitization	PASS
EP 3.1.9	European Pharmacopoeia 3.1.9 silicon elastomer for closures and tubing	PASS
ISO 10993-4	Hemolysis test – Autian method	PASS
ISO 10993-10	Kligman maximisation – Test for irritation and delayed type hypersensitivity	PASS
USP <87>	In vitro cytotoxicity test	PASS
ISO 10993-5	Quantitative MEM elution– ISO. Biological reactivity part 5, in vitro cytotoxicity	PASS
USP <381>	Physicochemical tests on elastomeric closure materials	PASS
ISO 10993-6	Short term intramuscular test	PASS
USP <88>	USP Class VI summary certificate	PASS
USP <85>	Limulus Amebocyte Lysate (LAL) Bacterial Endotoxin Assay	REPORT
USP <788>	USP Particulate/ Microscopic particulate count analysis test	REPORT

5b USP <88> Biological Reactivity Tests, *In Vivo*, Post Gamma Irradiation samples

USP Class VI Plastics Test assesses the potential toxicity of a given test article systemically, intracutaneously and through implantation.

Samples of the Pumpsil were gamma irradiated at 45 – 55kGY and tested in accordance with USP32, NF 27, <88>, biological reactivity tests, *In Vivo*. This included the immersion of the test articles in the following solutions: USP 0.9% NaCl, cottonseed oil, 1 in 20 ethanol in NaCl solution and polyethylene glycol 400 at 70C for 24 hours.

Results: *Pumpsil extracts and implants showed no toxicity, therefore Pumpsil passed USP Class VI testing.*

5c USP <87> Biological Reactivity Tests, *In Vitro*, Post Gamma Irradiation samples

USP Biological reactivity tests, In Vitro, post sterilisation samples Samples of Pumpsil were gamma irradiated at 45–55kGy and tested in accordance with USP43, NF38, , Biological reactivity tests, In Vitro. The biological reactivity of a mammalian monolayer, L929 mouse fibroblast cell culture in response to Pumpsil was determined. Samples of Pumpsil tubing, positive control (rubber) and negative control articles were prepared at 37°C for 48 hours. Biological reactivity was rated on a scale ranging from Grade 0 (no reactivity) to Grade 4 (severe reactivity).

Results: *No reactivity (grade 0) was exhibited by the cell cultures when exposed to Pumpsil. Therefore Pumpsil is not cytotoxic and passed the requirements of USP biological reactivity tests.*

5d ISO 10993-4 Hemolysis

The hemolysis test assesses the potential for indirect contact of a given sample with blood to cause the rupture of erythrocytes (red blood cells).

Pumpsil were tested in accordance with ISO 10993-4, 2002, Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions with Blood.

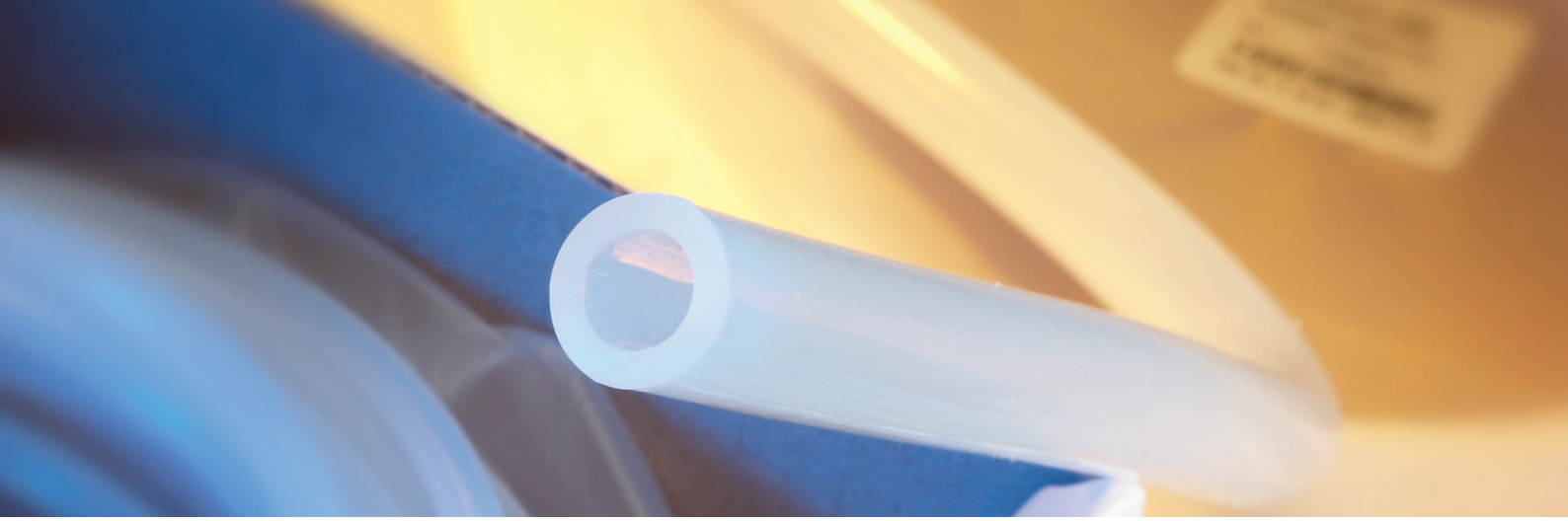
Results: *Per ISO 10993-4, Pumpsil is considered non-hemolytic.*

5e ISO 10993-6 Short term Intramuscular Implantation Test

The purpose of this test is to evaluate the solid material in direct contact with living tissue.

Pumpsil was tested in accordance with ISO 10993-6. Samples of Pumpsil (1mm x 1mm x 10mm) and the negative control plastics were evaluated. The test sites were examined for inflammation, encapsulation, necrosis, haemorrhage and discolouration macroscopically.

Results: *Pumpsil did not demonstrate any difference as compared to the control implant sites when in contact with tissue for two weeks. Therefore Pumpsil passed the requirements for ISO 10993-6.*



5f ISO 10993-10 Kligman Maximisation Test

The purpose of this test is to detect the allergenic potential of a test article.

Pumpsil was tested in accordance with ISO 10993-10. Samples of Pumpsil were extracted in USP 0.9% NaCl for injection and cottonseed oil at 70C for 24 hours and then injected intradermally. After two weeks, an additional topical application was introduced to the site of intradermal injections.

Results: *The sites that were exposed to the test articles and negative control showed no signs of erythema or edema. Therefore, Pumpsil is deemed not to contain any allergy.*

5g ISO 10993-5 Biological evaluation of medical devices- part 5: tests for in vitro cytotoxicity

SO 10993-5 Biological evaluation of medical devices—tests for In Vitro cytotoxicity. The biological reactivity of a cell culture, in response to extracts from Pumpsil was determined. The maintenance medium on the cell cultures was replaced by extracts of Pumpsil, or control article. The cell cultures were incubated for 48 hours at 37C ±1°C. Biological reactivity was evaluated by a photo spectrometer at 450nm wavelength.

Results: *Pumpsil showed no signs of cytotoxic activity. Therefore Pumpsil passed the requirements of ISO 10993-5.*

5h USP <381> Elastomeric closures for injections - physiochemical tests

Extracts of Pumpsil were prepared according to the requirements of USP 32, NF 27, Chapter 381 as directed under physiochemical tests. The results of the tests are summarised in *Table 3*.

Results: *Based on the evaluation criteria mentioned below, Pumpsil tubing meets the requirements of the USP <381> section physiochemical tests.*

Table 3: List of physiochemical tests that form USP <381>

Test	Test Result	Evaluation Criteria (Type One closures)	Result
Appearance of solution	0.09NTU	< 6NTU < less intense than matching fluid O	PASS
Acidity or alkalinity	0.1ml NaOH to produce blue colour	< 0.3ml NaOH to produce a blue colour	PASS
Heavy metals	Less intense colour than standard solution	Standard solution containing 2ppm Lead (Pb)	PASS
Reducing substances	0.2ml	< 3ml	PASS
Absorbance	0.1763ppm	< 0.2ppm	PASS
Extractable Zinc	0.0021ppm	< 5ppm	PASS
Ammonium	Less intense colour than standard solution	Standard solution not more than 2ppm of Ammonium present.	PASS
Volatile Sulfides	No black stain	Less intense colour than standard solution	PASS

5i ISO 10993-11 guidelines for the Systemic Injection test

The purpose of the systemic injection study is to screen test articles extracts for potential toxic effects as a result of a single dose systemic injection. Samples of Pumpsil tubing were extracted using 0.9% sodium chloride for injection, cottonseed oil, 1 in 20 ethanol in NaCl solution or polyethylene glycol 400 at 70C for 24 hours.

Results: *Pumpsil meet the requirements of ISO 10993-11 guidelines for the systemic injection test.*

5j ISO 10993-10 guidelines for intracutaneous Injection test

The intracutaneous test is designed to evaluate local responses to the extracts of Pumpsil following intracutaneous injection. Pumpsil tubing is extracted using 0.9% NaCl for injection, cottonseed oil, 1 in 20 ethanol in NaCl solution or polyethylene glycol 400 at 70C for 24 hours.

Results: *Pumpsil meet the requirements of ISO 10993-10 guidelines for the intracutaneous injection test.*

5k USP <85> Limulus amebocyte lysate (LAL) bacterial endotoxin assay

USP Limulus amebocyte lysate (LAL) bacterial entoxin assay Endotoxins are lipopolysaccharide complexes in gram negative bacterial cell walls. The limulus ameobocyte lysate (LAL) Kinetic Chromogenic assay is used to determine the quantitative level of endotoxin present within our products. Pumpsil tubing was tested in accordance to the requirements of USP 85. Pumpsil (22.0 cm^2 surface area) was extracted in 50mL of LAL reagent water at room temperature for 60 minutes. LAL-lysate was added to each sample well on an automated microtiter plate and then transferred to a plate reader where the absorbance of each well was read at 405 nm and 37 °C at set timepoints. A standard curve was generated using software based on reaction time versus endotoxin concentration which was then compared to the endotoxin concentration and reaction time of the sample extract.

Results: *Accusil extracts had an EU/ml value of <0.011 EU/cm^2 which is comparable to the levels of endotoxin observed in water for injection.*

5l USP <788> Particulate testing/ microscopic particulate count analysis test

This test is used to determine a level of particulates measuring 10 micron (µm) or less and 25 micron (µm) that may be present in any given drug product.

A length of Pumpsil tubing was filled with low particulate water and shaken 20 times. The extraction fluid was then recovered and the particles were measured using light obscuration.

Results: *Extracts from a Pumpsil tube contained 1187 particles > 10µm and 8 particles > 25µm*

5m European Pharmacopoeia 3.1.9

Extracts of Pumpsil were prepared in accordance with the requirements of European Pharmacopoeia, 2009, Chapter 3.1.9 Silicone elastomer for closures and tubing. The results of tests are summarised in *Table 4*.

Results: *Based on the results of the tests, Pumpsil tubing meets the requirements of EP 3.1.9 section Physiochemical tests.*

Table 4: List of physiochemical tests that form EP 3.1.9- Silicone elastomer for closures and tubing

Test	Test result	Evaluation criteria	Result
Appearance of solution	Clear, 1.344NTU	Clear, Turbidity < RS I (3NTU)	PASS
Acidity or alkalinity	2.5ml 0.01M NaOH (blue colour) 1.0ml 0.01M HCl (yellow changes to orange)	≤ 2.5ml of 0.01M NaOH change to blue ≤ 0ml of 0.01M HCl change to yellow to orange	PASS
Relative density	1.16 g/ml	1.05 – 1.25 g/ml	PASS
Reducing substances	0.8ml	Diff. between sample and blank ≤ 1.0ml	PASS
Substances soluble in hexanes	0.32%	3%	PASS
Volatile matter	0.17%	< 2 % (Pt cured)	PASS
Mineral oils	Less fluorescence than 1ppm standard	Less fluorescence than 1ppm standard	PASS
Phenylated compounds	Max absorbance < 0.009A.u.	Absorbance < 0.4A.u. (between 250 and 340nm)	PASS
Platinum	Less coloured than Pt reference (30ppm)	Less coloured than Pt reference (30ppm)	PASS

6. Extractables testing

Sections of Pumpsil tubing were subjected to extraction in multiple solvents at controlled temperatures. The test materials was extracted in a 6cm^2 : 1ml surface area to volume ratio. The solvent extracts were then analysed for volatile, semi volatile, non-volatile and metallic extractables using High pressure liquid chromatography – diode array detector - mass spectrometry (HPLC-DAD/MS), Direct injection gas chromatography – mass spectrometry (DI-GC/MS), Headspace gas chromatography – mass spectrometry (HS-GC/MS) and Inductively coupled plasma – mass spectrometry (ICP/MS).

33 elements were evaluated by ICP MS including those listed in ICH Q3D and USP 232 guidelines.

Results: *These studies have shown that there are low levels of extractables from the tubing extracts of 0.01N NaOH, 0.01M HCl and WFI solutions. WMFTS can provide further information and assistance in the evaluation of extractables data for risk assessment purposes.*

7. Conclusions

Pumpsil has been shown to pass a number of compendial and ISO testing summarised in this guide. For further information with full compliance statements and the test reports, please contact your WMFTS representative.

8. Downloadable validation document links

Section Number	Title of test	Link to the report (Click hyperlink to open)
5b/5e/5i/5j	ISO 10993-11/ISO 10993-10 – Systemic toxicity and intracutaneous injection USP<88> Biological reactivity tests, in vivo	*USP Class VI & ISO 10993 parts 6, 10 & 11*
5c/5g	USP<87> Biological reactivity tests, in vitro ISO 10993-5 – Quantitative MEM Elution in vitro cytotoxicity	*USP 87/ISO 10993-5 Qualitative MEM* *ISO 10993-5 Quantitative MEM*
5d	ISO10993-4 Hemolysis	*ISO 10993-4*
5f	ISO10993-10 Kligman Maximisation	*ISO 10993-part 10 (Kligman Maximisation)*
5h	USP <381> Elastomeric closures for injections	*USP 381*
5k	USP <85> Limulus ameobocyte lysate (LAL) bacterial endotoxin assay	*USP 85*
5l	USP <788> Particulate testing	*USP 788*
5m	European Pharmacopoeia 6.8, Chapter 3.1.9	*EP 3.1.9*
6	Extractables testing	*Extractables*

WATSON MARLOW Fluid Technology Solutions

BIOTECHNOLOGY AND PHARMACEUTICAL SOLUTIONS

WATSON MARLOW Pumps | WATSON MARLOW Tubing | Flexicon Liquid Filling | bioPURE | ASEPCO | AFLEX HOSE

Watson-Marlow Fluid Technology Solutions

Watson-Marlow Fluid Technology Solutions supports its customers locally through an extensive global network of direct sales operations and distributors

wmfts.com/global