WHITE PAPER

Detection of Counterfeit Biologic Drugs

Using 532 nm Handheld Raman

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INTRODUCTION

Counterfeit drugs are a rapidly growing global public health threat impacting both developing and developed countries. Counterfeits affect people of all ages, causing disability, injury, and even death. The magnitude of this problem is such that counterfeit medicine is estimated to have annual revenue of over $200 billion **[1]**, and cause hundreds of thousands of deaths worldwide every year **[2, 3]**. Moreover, since not all counterfeit activity is detected and/or reported, these figures are likely to be underestimates **[4]**.

In developed countries, major counterfeit targets tend to be “lifestyle medicines” including erectile dysfunction, hair loss, and weight management medications. However, as counterfeiting expanded, it also started targeting expensive medicines including cancer drugs, antivirals, and other biologic drugs **[5]**.

The biologic drugs (or biologics) are an ideal target group for counterfeiting due to their high cost that guarantees high margins. Recent examples of counterfeit biologics include incidents with counterfeit drugs Erythropoietin **[6]** and Avastin **[7]** that are used to treat kidney failure and cancer, respectively. In case of counterfeit Erythropoietin, vials were produced by the original manufacturer, Amgen, but were later up-labeled on the gray market denoting 40,000 U (units of activity) instead of their real content of 2,000 U. As for the counterfeit Avastin, the situation was even worse: the bogus batches came to the US from abroad and contained cornstarch, salts, acetone, and other potentially harmful chemicals, but no active pharmaceutical ingredient (API) to fight cancer.

Only portions of the counterfeit Erythropoietin and Avastin vials were discovered, indicating that the majority of these “drugs” must have been administered to patients. Yet a quick and simple check with an inexpensive RamTest-BIO™ Handheld Raman Identifier would have identified the falsified drugs at any stage of the distribution network, before they were given to patients.

WHY 532 NM RAMAN EXCITATION IS SUPERIOR FOR BIOLOGIC MOLECULES?

A winner of the prestigious Analytical Scientist Innovation Award in 2016 **[8]**, RamTest-BIO™ Handheld Raman Identifier (*Figure 1*) is specifically developed to enable best-in-class (among handhelds) performance for biologics identification, quantitation and structural characterization. The superior performance is achieved by combining the innovative optical design to minimize signal losses, 532 nm laser excitation to several-fold improve Raman signal strength per unit laser power (comparing to conventional 785 and 1064 nm excitations), and the state-of-the-art methodology to reduce impact of fluorescence on Raman measurements **[9]**.

Figure 1 *RamTest-BIO™ Handheld Raman Identifier.*

Attachments (bottom left) enable measurements of liquid / solid biologics ‘as is’; on a slide; or through containers: vials, bottles, syringes, jars, Petri dishes, etc.

Recent publications from Amgen **[10]** and Merck **[11]** have demonstrated that analytical methods, based on the 532 nm benchtop Raman technology, are highly effective for structural characterization of biologic drugs including secondary and higher order structures, as well as early detection of aggregation and/or structural degradation. Impressed by the demonstrated analytical performance of the bench-top 532 nm Raman, BioTools Inc. (Jupiter, FL, USA) has recently developed a "miniaturized" handheld version of this technology **[8]**. Similarly to its "older" benchtop ancestor, the new 532 nm handheld Raman is proving to be a powerful time- and cost-saving option for biologics structural characterization, quantitation in water, and/or counterfeit testing **[9]**.

Here are some of the benefits of the 532 nm handheld Raman for biologics characterization, including the counterfeit testing:

- **Several-fold faster analysis compared to conventional handheld Raman, improved analysis accuracy, and/or significantly reduced detection limits [9]**
    - This is because Raman signal intensity, per unit laser power, is inversely proportional to 4^{th} power of laser excitation wavelength, $I_{RAMAN} \sim (1/\lambda_{EX})^4$, where λ_{EX} = **532 nm**, **785 nm**, **1064 nm [12]**. Thus, 532 nm excitation directly results **in 5 and 16 times stronger Raman signal** (faster analysis, improved analysis accuracy, and/or reduced detection limits) comparing to conventional handheld Raman units utilizing 785 nm and 1064 nm excitation, respectively.
- **Best-in-class analysis of complex mixtures, accuracy of material identification (ID), and/or quantitation of analytes in aqueous solutions and/or most of organic solvents [9]:**
    - Unmatched combination of spectral resolution (~4 cm^{-1}) and spectral range (~120-4000 cm^{-1}), that is unachievable with commercial 785 and 1064 nm handheld Raman units due to engineering limitations
    - Superior quantitation of analytes including biologics in aqueous solutions using the ~3200-3400 cm^{-1} OH-stretch bands of water that are unattainable with the other handhelds currently available on market **[9]**
- **Non-destructive analysis, no need to open a container:**
    - Analyzes / IDs substances "as is," or directly through most of glass and plastic containers including clear, amber, blue, green, and others. Applicable containers include: vials, bottles, jars, plastic bags, blister packs, Petri dishes, etc. **[9]**
    - No contact or contamination with sample occurs and no liquid or any other substance is brought in contact with the sample during test, particularly important for delicate biologic molecules and/or small or trace amounts of sample
- **Broad applicability scope:**
    - Applicable to substances in essentially any physical state: powders, tablet/pill, crystals, chips, or fibers; neat liquids, solutions, gels, or suspensions
- **Superior Raman analysis business case, best performance-to-price ratio on market:**
    - In addition to the superior analytical performance, a 532 nm handheld Raman unit cost is up to ~2-fold lower than that of conventional 785 or 1064 nm handheld Raman. This is because the 532 nm optical components and light detectors are less expensive than those utilized for the conventional handheld Raman units based on 785 or 1064 nm laser excitation **[9]**

In addition to **SUPERIOR RAMAN ANALYSIS BUSINESS CASE**, the 532 nm handheld Raman enables non-destructive, fastest-in-class collection of a full Raman spectrum (including O-H, C-H, N-H, and N≡N stretches) in a **SINGLE MEASUREMENT** with, simultaneously, best-in-class spectral resolution among all the conventional 785 and 1064 nm Raman handhelds available on market today.

EXPERIMENTAL

All biologic drug samples were analyzed using a RamTest-BIO™ handheld Raman Identifier (BioTools, Inc., Jupiter, FL, USA) shown in *Figure 1*. The original drug samples were analyzed in non-invasive manner, directly through their original vials without opening. Thus, no contact or contamination with sample occurred and no liquid (or other substance) was brought into contact with the sample during test. This is of a particular importance for analysis of delicate biologic molecules and/or small amounts of sample.

All tests were run in the automated mode (requiring no prior knowledge of Raman spectroscopy), where all measurement parameters are automatically adjusted to optimize signal-to-noise ratio and minimize fluorescence, with remaining fluorescence background (if any) automatically subtracted.

RamTest™ software is CFR 21 compatible and comes with a USER-EDITABLE database of reference compounds.

TEST MEASUREMENT: COUNTERFEIT vs AUTHENTIC BIOLOGIC DRUGS

To imitate the accidents with counterfeit Erythropoietin and Avastin described in the Introduction, we took two different biologics from the 2014 Top-10 best-selling list (Table 1). First, we measured the spectra of both biologics “as is” to have a reference spectra of the authentic drugs. Then, we simulated the counterfeit Erythropoietin case with strongly diluted active pharmaceutical ingredient (API), and the bogus Avastin case with no API at all.

Table 1 **The 10 best-selling biologics** (Data Source: Genetic Engineering News and Drug Prescribing Information)

Rank	Biologic	Company	2014 Sales
1	Humira (adalimumab)	AbbVie	12.5 billion
2	Remicade (infliximab)	Johnson & Johnson and Merck & Co.	9.2 billion
3	Rituxan (rituximab, MabThera) includes sales of next generation version of Rituxan -Gazyva	Roche and Biogen Idec	8.6 billion
4	Enbrel (etanercept)	Amgen and Pfizer	8.5 billion
5	Lantus (insulin glargine)	Sanofi	7.2 billion
6	Avastin (bevacizumab)	Roche	6.9 billion
7	Herceptin (trastuzumab)	Roche	6.7 billion
8	Neulasta/Neupogen (pegfilgrastim)	Amgen and Kyowa Hakko Kirin	5.8 billion
9	Prevnar 13/Prevenar 13 and Prevnar/Prevenar (7-valent)	Pfizer	4.4 billion
10	Avonex (interferon beta-1a)	Biogen Idec	3.0 billion

Specifically, to imitate the Erythropoietin incident, we diluted both biologics 10-fold with a formulation buffer. To simulate the Avastin case, we utilized the formulation buffer (placebo) with no API in it. Figure 2 shows the spectra of the authentic product, diluted product/API, and the "no API" case, respectively**. In case of both biologics #1 and #2, it is quick, easy and unambiguous to differentiate the "counterfeit drugs" from the original ones.**

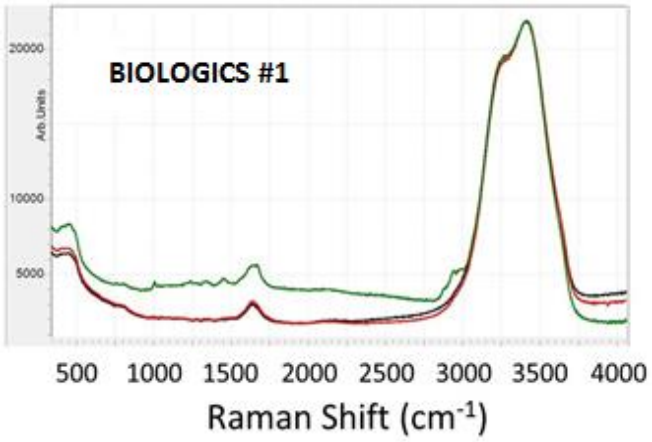

Figure 1. *Biologics #1 measured with RamTest-BIO™ handheld Raman identifier directly through the original vials.*

Original/authentic drug #1 (green), 10-times diluted drug to imitate a counterfeit drug with diluted API (red), and formulation buffer with no API to mimic counterfeit drug with no API case (black). Buffer is NOT subtracted.

NOTE: after buffer subtraction, the drug with 10-fold diluted API can also be easily distinguished from the drug with "no API" (data not shown).

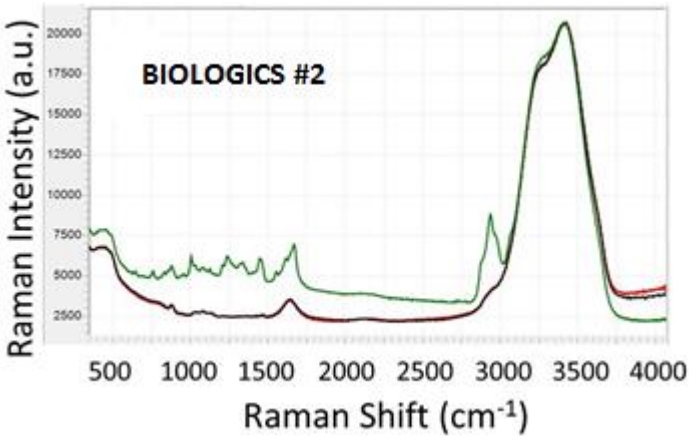

Figure 3. *Biologics #2 measured with RamTest-BIO™ handheld Raman identifier directly through the original vials.*

Original/authentic drug #2 (green), 10-times diluted drug to imitate a counterfeit drug with diluted API (red), and formulation buffer with no API to mimic counterfeit drug with no API case (black). Buffer is NOT subtracted.

NOTE: after buffer subtraction, the drug with 10-fold diluted API can also be easily distinguished from the drug with "no API" (data not shown).

This experiment demonstrates that the incidents with the use of counterfeit Erythropoietin and Avastin could have been easily prevented by executing a simple test with a RamTest-BIO™ handheld Raman Identifier (BioTools, Inc., Jupiter, FL, USA). This conclusion can likely be extrapolated to many other counterfeit medicine cases including but not limited to those involving biologics.

To even further improve efficiency of counterfeit biologics detection, BioTools Inc. (Jupiter, FL) has recently commercialized inexpensive drop coating deposition Raman (DCDR) technology **[13]**, sold as **"μ-RIM™ substrate"**. This μ-RIM™ substrate is compatible with RamTest-BIO™ Handheld Raman Identifier, as well as several other BioTools' portable and benchtop Raman instruments. With this technology, as low as 1-10 μL of biologics solution can be deposited onto a μ-RIM hydrophobic surface to dramatically (up to 30-1000 times) improve Raman (or FTIR) signal-to-noise ratio compared to that in the original solution. The signal-to-noise ratio improvement occurs as a sample precipitates into a pre-concentrated "coffee ring" that is formed as a result of the sample-to-substrate contact line pinning, sample evaporation, and capillary flow on the DCDR substrate's hydrophobic surface **[13]**. Moreover, the pre-concentration process produces biologics or protein deposits that are in a well-hydrated glassy-like state to essentially preserve the original native (biologically active) structural conformation of the sample **[14, 15]**.

CONCLUSIONS

Our analysis indicates that the non-conventional 532 nm handheld Raman is a highly attractive option for biologic drugs (or biologics) identification, characterization, and/or counterfeit testing. Specifically, we have demonstrated that the incidents of the type that occurred with the counterfeit Erythropoietin and Avastin can be easily prevented by a fast and simple test using a 532 nm RamTest-BIO^{TM} handheld Raman identifier (BioTools, Inc., Jupiter, FL, USA).

The 532 nm handheld Raman not only delivers a superior analysis or ID quality for biologics, but also dramatically improves business case(s) for both field- and laboratory-based Raman analysis comparing to conventional 785 and 1064 nm handheld Raman. Identified benefits include 50% reduction in instrument cost, up to 5-16 times faster analysis, superior performance in water and most of organic solvents, ability to non-invasively analyze samples through a large variety of glass and plastic containers (including amber), best-in-class combination of spectral range and spectral resolution, as well as reduced detection limits with improved analysis accuracy **[9]**.

In addition to offering fastest- and most-reliable-in-class biologics ID, the 532 nm handheld Raman also provides best-in-class accuracy of quantitative analysis in aqueous solutions. This is achieved by utilizing the ca. 3200-3400 cm^{-1} OH-stretch bands of water as internal Raman intensity and/or cross-section standards **[9]**. These bands are only attainable with the 532 nm handheld Raman, but not with today's 785 and 1064 nm handheld Raman units, due to the optical component / engineering limitations.

In summary, 532 nm handheld Raman technology can effectively identify counterfeit drugs at any stage of the distribution network, before the drugs are given to patients. Due to its superior analytical performance for biologics, user-friendliness, and relatively low cost-per-unit, the 532 nm handheld Raman is a powerful time- and money-saving tool for essentially every player in drug market including pharmaceutical companies, regulatory and law-enforcement agencies, upstream and downstream distributors, hospitals, receiving pharmacies and end-user doctor offices.

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GLOSSARY

Biologics – biologic drugs, new type of drugs that are produced by means of modern biotechnology. These drugs revolutionized treatment of many serious deceases including cancer, Alzheimer, diabetes, arthritis, HIV/AIDS, and others

API – Active pharmaceutical ingredient

U – Unit of activity. Unit of activity typically describes enzyme catalytic activity, where a unit (U) refers to the amount of enzyme that catalyzes the conversion of 1 micromole (μmole) of substrate per minute.

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ADDITIONAL INFORMATION

Resources:

RamTest Flyer: http://www.btools.com/assets/ramtest_flyer_biotoolsv2.pdf

BioTools Products: http://www.btools.com/products.html

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