A Rapid iMethod™ Test for the Screening of Antibiotics in Kidney Juice, Serum, Milk, and Honey

Meat, dairy, fish, honey, and other animal products need to be routinely monitored for veterinary drug residues that are used to fight disease and infection in animals, but harmful to humans if present upon ingestion. However, with wide availability, variable legislation and variable animal husbandry practices from country to country, high drug residues levels in produce continues to be an issue. Over-use and / or inappropriate use of antimicrobial drugs can lead to increased antimicrobial resistance, reducing the ability to fight human infection. On top of this, exposure to such drugs can affect human reproduction, cause cancer and have a toxic effect.

The following information outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Antibiotic Screening when using a 4000 QTRAP® LC/MS/MS System, utilizing an MRM triggered EPI workflow that can be applied to a library for confirmation. Please note that the 169 compound antibiotic screening library is sold separately and not included with this iMethod™ Test.

The iMethod™ Test screens for several classes of antibiotics including: beta-lactam, tetracycline, sulfonamide, macrolide, amphenicol, fluoroquinolone and flunixin. A list of compounds is shown below in Table 1. The method uses positive ion and negative ion acquisition modes to achieve maximum sensitivity. This iMethod™ Test includes an MRM catalogue containing 93 compounds with up to three transitions per compound that can be used to create customized screening or quantification tests.

Example sample preparation protocols are included for use with beef kidney juice and serum, milk, or honey, that consist of homogenization, extraction with acetonitrile, solid phase extraction, centrifugation, and filtration, with an injection from water.

The separation consists of the use of a methanol / water gradient with formic acid with separation on a Phenomenex Gemini C18 column. An example chromatogram of the separation achieved is shown in Figure 1.

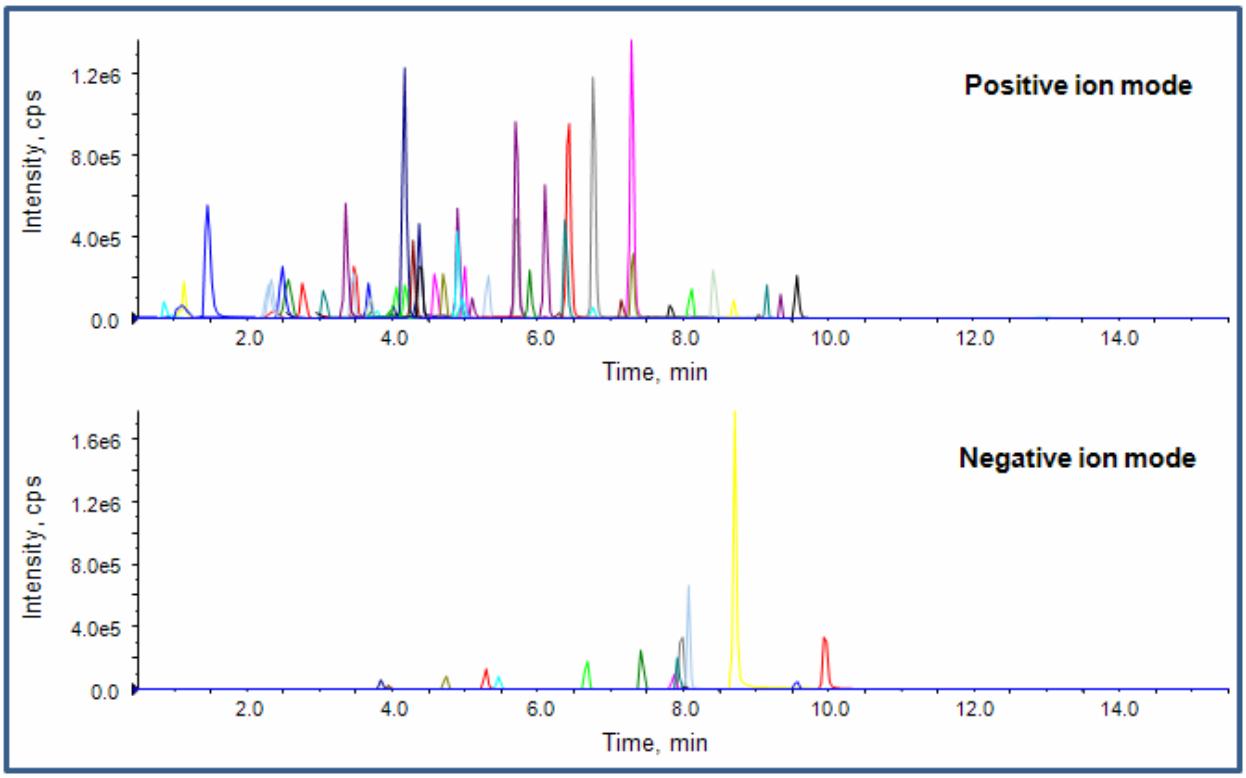

Figure 1: Extracted Ion Chromatogram of antibiotics standard mixture working solution (at 200 ng/mL), obtained on a 4000 QTRAP® LC/MS/MS System

Table 1. List of compounds in the iMethod Test for Veterinary Antibiotic Screening Version 1.1 MRM Catalogue. Compounds in Blue are run in Negative mode.

Compounds	Compounds, cont	Compounds, cont	Compounds, cont
2-mercaptobenzimidazole	Doxycycline*	Sulfadimethoxine*	Sulfadiazine*
2-quinoxalinecarboxylic acid	Enoxacin*	Sulfadoxine*	Sulfaguanidine*
6-phenyl-2-thiouracil	Enrofloxacin	Monensin	Sulfamerazine*
Albendazole	Erythomycin*	Naproxen	Sulfamethazine*
Albendazole Amino Sulfone	Etodolac	Narasin	Sulfamethazine-d4*†
Albendazole Sulfone	Fenbendazole	Nicarbazin	Sulfamethizole*
Albendazole Sulfoxide	Fenbendazole Sulfone	Niclosamide	Sulfamethoxazole*
Amoxicillin*	Fenbendazole Sulfoxide	Norfloxacin*	Sulfamethoxypyridazine*
Ampicillin*	Florfenicol Amine*	Novobiocin	Sulfanilamide*
Bacitracin	Florfenicol*	Ofloxacin*	Sulfanitran
Carbadox*	Flumequine	Orbifloxacin*	Sulfapyridine*
Ceftiofur	Flunixin*	Oxolinic acid*	Sulfaquinoloxaline*
Chloramphenicol*	Ibuprofen	Oxyphenbutazone	Sulfasalazine*
Chlorotetracycline*	Indomethacin	Oxytetracycline*	Sulfathiazole*
Ciprofloxacin*	Ipronidazole-OH	Penicillin G*	Sulfisoxazole*
Clindamycin*	Josamycin*	Phenylbutazone	Tetracycline*
Cloxacillin*	Ketoprofen	Ractopamine	Tetramisole*
Danofloxacin*	Lasalocid (pos and neg)	Rafoxanide	Thiabendazole
Decoquinate	Lincomycin*	Ronidazole	Thiamphenicol*
Desethylene Ciprofloxacin*	Lomefloxacin*	Sarafloxacin*	Tilmicosin
Diclofenac	Mebendazole	Spiramycin*	Tolfenamic acid
Difloxacin*	Metronidazole	Sulfacetamide*	Triclabendazole
Dimetridazole	Minocycline*	Sulfachloropyridazine*	Tylosin*
Dipyrone			

** denotes compounds in iMethods™ Test for Veterinary Antibiotics Screening, Version 1.1 for Bovine Kidney Juice*
† denotes internal standard

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 4000 QTRAP® or QTRAP® 5500 LC/MS/MS System
- A Shimadzu Prominence 20A LC System with Reservoir tray and bottles, System controller CBM-20A, 100 µL mixer, 2 Isocratic pumps LC-20AD, 3 Channel degasser Autosampler SIL-20AC, Column oven CTO-20AC **or** Agilent 1200 LC system with Binary pump G1312B, Vacuum degasser G1379B (without static mixer), Well plate Auto sampler G1367C , and Thermostated Column oven G1316B.
- LC/MS Grade Water, Methanol, Ammonium Formate
- A Phenomenex 3.0 µm 110Å, Gemini C18, 50 x 2 mm HPLC column
- 1.5 mL Eppendorf Tubes
- A Centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- Pipettes and standard laboratory glassware
- Antibiotic Standards (www.sigmaaldrich.com)
- Please note the Phenomenex Gemini C18 HPLC column comes standard with this iMethod™ Test Kit. Also, that this method can be run on other HPLC systems, given that they

are supported for use by Cliquid® Software and retention times are updated according to the configuration used.

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the screening of antibiotics, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The purchase and use of certain chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements.

Legal Acknowledgements/Disclaimers

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions It is the responsibility of the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

The suppliers identified in this document are provided for information purposes only and is not intended to be an exhaustive representation of all manufacturers or suppliers of the referenced product. AB SCIEX makes no warranties or representations as to the fitness or the continued fitness of a specific product by any of the manufacturers/suppliers referenced herein or the supplier. AB SCIEX assumes no responsibility or contingent liability, including indirect or consequential damages, for any use to which the purchaser may put the referenced suppliers' products, or for any adverse circumstances arising therefrom.

Ordering Information

Product Name	Part Number
iMethod™ Test for Antibiotic Screening V.1.1 for Cliquid® Software	5008145

For Research Use Only. Not for use in diagnostic procedures.

Publication number: 1260310-01

Headquarters
353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

International Locations
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices