GXT INSTRUKCIJA NR. 1

1 Introduction—Use or Function

This chapter provides an overview of the GeneXpert Dx system. The topics are:

Note GeneXpert Dx software version 4.8 supports the Microsoft Windows 7 operating system. Should you need any assistance, please contact your regional Cepheid Technical Support center.

1.1 Intended Use

The GeneXpert Dx system automates and integrates sample preparation, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time Polymerase Chain Reaction (PCR). The system is suited for *in vitro* diagnostic applications that require hands-off processing of patient samples (specimens) and provides both summarized and detailed test results data in tabular and graphic formats.

The GeneXpert Dx System is designed exclusively for the use of Cepheid Xpert® assay applications. It allows a laboratory healthcare professional to run tests on the GeneXpert Dx platform using predefined assays.

1.2 Terms Used for System Descriptions

In this manual, the following terms are used to describe the GeneXpert Dx systems:

- GeneXpert Dx system refers to the complete system including the computer, GeneXpert instrument and barcode scanner.
- GeneXpert instrument refers only to the components used to process the samples. See Figure 1-1, Figure 1-2, Figure 1-3 and Figure 1-4 for examples of GeneXpert instruments.

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1.3 Models of GeneXpert Instruments

There are three different GeneXpert R1 instruments:

- The GeneXpert GX-I instrument consists of one module (or one site) to process one sample. Up to four GeneXpert GX-I instruments can be connected to one computer.
- The GeneXpert GX-IV instrument consists of up to four modules. Each module processes one sample. Up to four GeneXpert GX-IV instruments can be connected to one computer.
- The GeneXpert GX-XVI instrument consists of up to sixteen modules. Each module processes one sample.

There are four different models of GeneXpert R2 instruments:

- The GeneXpert GX-I instrument consists of one module (or one site) to process one sample. Up to four GeneXpert GX-I instruments can be connected to one computer.
- The GeneXpert GX-II instrument consists of one or two modules. Each module processes one sample. Up to four GeneXpert GX-II instruments can be connected to one computer.
- The GeneXpert GX-IV instrument consists of up to four modules. Each module processes one sample. Up to four GeneXpert GX-IV instruments can be connected to one computer.
- The GeneXpert GX-XVI instrument consists of up to sixteen modules. Each module processes one sample. One GeneXpert GX-XVI instrument can be connected to one computer.

For purposes of this document, the GeneXpert Dx systems function identically and will not be identified as R1 or R2 unless there is a specific difference noted.

1.4 System Components

The components of the GeneXpert Dx systems are as follows:

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- **GeneXpert Instrument**—Accepts the GeneXpert cartridges that are loaded into the instrument, lyses the samples in the cartridges, releases the nucleic acids, and amplifies the target sequences. Because the system allows control of the modules independently, different samples can be processed using different assay definitions in the same instrument at the same time.
- **Desktop or Laptop Computer**—Allows you to run the GeneXpert Dx system software and hosts the GeneXpert Dx system results database. The software allows the selection of assay definitions, monitoring of test process, viewing results, and exporting of selected data to downstream software, such as Microsoft Excel, for additional analysis. The software also allows the archiving and retrieval of the results data and management of the database. Cepheid Link connectivity is provided to enable cartridge traceability.
- **Barcode Scanner**—Facilitates data entry in the system.

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1.5 GeneXpert Cartridges

- The samples are prepared and processed in single-use, assay-specific GeneXpert cartridges (see Figure 1-5). The sample and applicable reagents are inserted into a cartridge and then the cartridge is loaded into one of the available instrument modules.
- The cartridges are not supplied with the system and must be purchased separately. For ordering information, contact Cepheid. See the Technical Assistance section in the Preface for the contact information.

Figure 1-5. GeneXpert Cartridge

1.6 GeneXpert Dx Software

The GeneXpert Dx software is installed on the supplied computer and can accommodate a variety of applications. This section describes the software features that are for *in vitro* diagnostic use (Figure 1-6):

- **Administrative tasks**—Configure the system to accommodate the organization's preferences, define system users and set up permissions (access privileges), import and delete *in vitro* diagnostic assay definitions, generate external control trend reports, and manage the test data in the database.
- **Test tasks**—Create and start an *in vitro* diagnostic test, stop a test in progress, monitor a test in progress, view the test results, edit test information, and generate test reports.
- **Maintenance tasks**—Perform various maintenance tasks which include using the Module Reporters tool and Plunger controls for cleaning the module plungers, performing a self-test manually for troubleshooting and checking the calibration and test counts, and utilizing commands for opening a module door or updating the EEPROM.

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For a summary of the workflows for in vitro diagnostic use, see Section 1.7, Workflow Overview.

INFO TYRIMUI NR. 1

DATASHEET

Xpert® **Xpress** Flu/RSV

Xpert® Xpress Flu/RSV

Test Reagent Kit	Xpert **Xpress** Flu/RSV		
Catalog Number	XPRSFLU/RSV-CE-10		
Technology	Real-time RT-PCR		
Targets	**Flu A**	**Flu B**	**RSV**
	Genes encoding matrix protein, PB2 and PA	Genes encoding matrix protein and non-structural protein	Gene encoding nucleocapsid of RSV A and RSV B
Batch or On-Demand	On-Demand		
Minimum Batch Size	1		
Sample Types	Nasopharyngeal Swab and Nasal Swab		
Sample Extraction	Automated/Integrated		
Precision Pipetting	Not required		
10 **TAT**	As early as 20 mins for positives* and about 30 minutes for negatives		
Hands-On Time	<1 min		
Controls: Process	Sample Processing Control		
Controls: Probe Function/ Detection	Probe Check Control		
Nasopharyngeal (NP) Swab	**Flu A**	**Flu B**	**RSV**
Positive Percent Agreement	98.1%	100.0%	98.4%
Negative Percent Agreement	98.8%	99.1%	99.3%
Nasal Swab (NS)	**Flu A**	**Flu B**	**RSV**
Positive Percent Agreement	98.9%	98.4%	98.2%
Negative Percent Agreement	97.5%	99.3%	99.1%
Sample Storage	2-30 °C for up to 24 hours or 2-8 °C for up to 7 days in transport medium		
Kit Storage	2-28 °C until the expiration date provided on the label		
Commercial Controls	Refer to Product Insert (301-6580, Rev C.) or contact Cepheid Technical Support		

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* With early assay termination (EAT) for positive Flu or for positive RSV only. Reporting negatives and combined Flu and RSV results in 30 minutes. Refer to Product Insert Xpert Xpress Flu/RSV CE-IVD 301-6580, Rev C.

CE IVD *In Vitro* Diagnostic Medical Device Not available in all countries. Not available in the United States.

CORPORATE HEADQUARTERS
904 Caribbean Drive
Sunnyvale, CA 94089 USA
TOLL FREE +1.888.336.2743
PHONE +1.408.541.4191
FAX +1.408.541.4192

EUROPEAN HEADQUARTERS
Vira Solelh
81470 Maurens-Scopont France
PHONE +33.563.82.53.00
FAX +33.563.82.53.01
EMAIL cepheid@cepheideurope.fr

www.Cepheidinternational.com

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REAGENTŲ INSTRUKCIJA NR.1

Xpert® C. difficile BT

In Vitro Diagnostic Medical Device

1 Proprietary Name

Xpert® *C. difficile* BT

2 Common or Usual Name

Xpert® *C. difficile* BT Assay

3 Intended Use

The Cepheid Xpert *C. difficile* BT Assay, performed on the Cepheid GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test for rapid detection of *C. difficile tcdB* (toxin B gene), *cdt* (binary toxin gene), and a deletion of a nucleotide at position 117 of the *tcdC* gene from unformed (liquid or soft) stool specimens collected from patients suspected of having *Clostridium difficile* infection (CDI). The Xpert *C. difficile* BT Assay is intended as an aid in the diagnosis of CDI and detection of strains potentially associated with more severe disease. The test utilizes automated real-time polymerase chain reaction (PCR) to detect *tcdB*, *cdt*, and the *tcdC* deletion at base 117 associated with the ribotype 027 strain. Binary toxin is produced by a limited number of *C. difficile* strains, including the 027 strain. Binary toxin together with *tcdB* detection is often an indicator of more severe disease or recurrence of disease. Isolates of *C. difficile* that are negative for *tcdB* but contain binary toxin genes alone may produce symptoms similar to toxigenic *C. difficile* strains but the clinical significance of such strains is currently uncertain. Concomitant culture is necessary only if further typing or organism recovery is required.

4 Summary and Explanation

C. difficile is a Gram-positive, spore-forming, anaerobic rod that was first linked to disease in 1978.[1]

CDI ranges from mild diarrhea to severe life-threatening pseudomembranous colitis.[2] Mature colonic bacterial flora in a healthy adult is generally resistant to *C. difficile* colonization.[3] However, if the normal colonic flora is altered, resistance to colonization by other bacterial species, such as *C. difficile*, is lost. The most common risk factor for developing CDI is exposure to antibiotics.[4] *C. difficile's* primary virulence factor is cytotoxin B.[5] The genes coding for toxin A (*tcdA*; the enterotoxin) and toxin B (*tcdB*) are part of the pathogenicity locus (PaLoc).[6,7] Most pathogenic strains are toxin A-positive, toxin B-positive (A+B+) strains, although toxin A-negative, toxin B-positive (A-B+) variant isolates have been recognized as pathogenic.[8] Some strains of *C. difficile* also produce an actin-specific ADP-ribosyltransferase called CDT or binary toxin. The binary toxin locus contains two separate genes (*cdtA* and *cdtB*) and is located outside the PaLoc.[9-11]

CDI diagnosis traditionally has been based either on the detection of toxin B directly in stool (the cell culture cytotoxicity neutralization [CCCN] test) or on culture of the organism followed by determination of toxin B production by the isolate (toxigenic culture). Both the CCCN test and toxigenic culture are labor intensive but are still considered to be the "gold standards" because of the specificity of the former and the sensitivity of the latter.[12,13] Several rapid enzyme immunoassays have been developed for detection of toxin A and B; however, these tests have reduced sensitivity and specificity compared to the CCCN test. PCR methods for the detection of genes associated with toxin A and/or toxin B production have been developed and show high sensitivity and specificity as compared to toxigenic culture.[14]

In addition to toxin A and B, recent literature suggests a link between the production of binary toxin and both disease severity and outcome. Bauer et al.[15] showed the presence of binary toxin genes in toxigenic isolates in 23% of the CDI cases in Europe. Binary toxin produced by *cdt* genes is frequently observed in *C.difficile* strains associated with increased severity of CDI. Binary toxin belongs to the family of ADP-ribosylating toxins and consists of *cdtA* genes, the enzymatic ADP-ribosyltransferase, which modifies actin, and *cdtB*, which binds to host cells and translocates the product of *cdtA* into the cytosol. Multiple clinical studies indicate an association between the presence of binary toxin genes in *C. difficile* and increased 30-day CDI mortality independent of PCR ribotype. There is also literature showing that subjects having severe CDI, fulminant colitis, and/or recurrent CDI are infected more frequently with *C. difficile* ribotypes carrying the genes for binary toxin production (*cdtA/cdtB*) than those without these complications.[16,17]

REAGENTY INSTRUKCIJA NR.1

19 Analytical Performance

19.1 Analytical Specificity

Fifty-five (55) strains were collected, quantitated and tested using the Xpert *C. difficile* BT Assay. The strains originated from the American Type Culture Collection (ATCC), Culture Collection University of Göteborg (CCUG), German Collection of Microorganisms and Cell Cultures (DSMZ), the Centers for Disease Control and Prevention (CDC), the Institute of Public Health, Maribor, Slovenia and Swedish Institute for Infectious Disease Control (SMI).

Of the bacterial species that were tested, ten (10) non-toxigenic *C. difficile* strains and eleven (11) non-*C. difficile Clostridium* species were included. The organisms tested were identified as either Gram-positive (37) or Gram-negative (18). The organisms were further classified as aerobic (24), anaerobic (29) or microaerobic (2).

Each strain was tested in triplicate at concentrations ranging from $1.1x10^8$ to $2.2x10^{10}$ CFU/swab. Positive and negative controls were included in the study. Under the conditions of the study, all isolates were reported **Toxigenic C. diff NEG; Binary Toxin NEG; 027 NEG** (Table 7). The analytical specificity was 100%.

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An additional series of non-*difficile Clostridium* species were tested to demonstrate the specificity of the binary toxin assay.

Table 7. Binary Toxin Gene Specificity Study Results

Genus	Species	Number Tested	Toxin A/B	Binary Toxin
Clostridium	*aldenense*	2	neg	neg
Clostridium	*aminovalericum-like*	2	neg	neg
Clostridium	*baratii*	2	neg	neg
Clostridium	*bartletti*	1	neg	neg
Clostridium	*bifermentans*	2	neg	neg
Clostridium	*bolteae*	2	neg	neg
Clostridium	*butyricum*	2	neg	neg
Clostridium	*cadaveris*	2	neg	neg
Clostridium	*celerecrescens*	2	neg	neg
Clostridium	*citroniae*	2	neg	neg
Clostridium	*clostridioforme*	2	neg	neg
Clostridium	*cochlearium*	1	neg	neg
Clostridium	*colicanis*	2	neg	neg
Clostridium	*disporicum*	1	neg	neg
Clostridium	*fallax*	2	neg	neg
Clostridium	*glycolicum*	2	neg	neg
Clostridium	*hastiforme*	1	neg	neg
Clostridium	*hathewayi*	2	neg	neg
Clostridium	*hylemonae*	2	neg	neg
Clostridium	*innocuum*	2	neg	neg
Clostridium	*lactatifermentans*	2	neg	neg
Clostridium	*lavalense*	1	neg	neg
Clostridium	*limosum*	2	neg	neg
Clostridium	*mangenotii*	1	neg	neg
Clostridium	*mayombei-like*	1	neg	neg
Clostridium	*novyi*	2	neg	neg
Clostridium	*paraputrificum*	2	neg	neg
Clostridium	*perfringens*	2	neg	neg

REAGENTŲ INSTRUKCIJA NR.2

Xpert® MTB/RIF Ultra

For *In Vitro* Diagnostic Use

Proprietary Name

Xpert® MTB/RIF Ultra

Common or Usual Name

Xpert MTB/RIF Ultra Assay

A. Intended Use

The Xpert MTB/RIF Ultra Assay, performed on the GeneXpert Instrument Systems, is a semi-quantitative, nested real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for the detection of *Mycobacterium tuberculosis* (MTB) complex DNA in unprocessed sputum samples or concentrated sediments prepared from induced or expectorated sputum. In specimens where *Mycobacterium tuberculosis* complex is detected, the Xpert MTB/RIF Ultra Assay can also detect rifampin-resistance associated mutations of the *rpoB* gene.

The Xpert MTB/RIF Ultra Assay is intended for use with specimens from patients for whom there is clinical suspicion of tuberculosis (TB) and who have received no antituberculosis therapy, or less than 3 days of therapy in the last 6 months. This test is intended as an aid in the diagnosis of pulmonary tuberculosis when used in conjunction with clinical and other laboratory findings.

B. Summary and Explanation

Globally, about 2 billion people are infected with MTB.[1] In 2015, 10.4 million people developed active disease, and 1.4 million people lost their lives to the illness.[2] The route of transmission of pulmonary TB is through the air, which makes this a highly transmissible disease. Given the infectious nature of pulmonary TB, fast and accurate diagnosis is an important element of TB treatment and control.

Treatment involves prolonged administration of multiple drugs and is usually highly effective. However, *M. tuberculosis* strains may become resistant to one or more of the drugs, making cure much more difficult to achieve. Four common first-line drugs used in anti-tuberculosis therapy are isoniazid (INH), rifampin (also known as rifampicin, RIF), ethambutol (EMB), and pyrazinamide (PZA). As documented by World Health Organization, RIF resistance is rarely encountered by itself, and usually indicates resistance to a number of other anti-TB drugs.[3] It is most commonly seen in multi-drug resistant (MDR-TB) strains (defined as resistant to both RIF and INH) and has a reported frequency of greater than 95% in such isolates.[4,5,6] Resistance to RIF or other first-line drugs usually indicates the need for full susceptibility testing, including testing against second-line agents.

Molecular detection of TB and *rpoB* gene mutations associated with RIF resistance greatly reduces the time to diagnosis of both drug-susceptible and MDR tuberculosis. With the Xpert MTB/RIF Ultra Assay, this can be accomplished in unprocessed sputum samples and in prepared sediments in less than 80 minutes. The rapid detection of MTB and RIF resistance allows the physician to make critical patient management decisions regarding therapy during a single medical encounter.

C. Principle of the Procedure

The GeneXpert Instrument Systems integrate and automate sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and melt peak detection. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on patient samples and viewing the results. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual.*

Xpert MTB/RIF Ultra Assay includes reagents for the detection of MTB and RIF resistance and a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor for the presence of inhibitor(s) in the PCR reaction and subsequent melt peak detection. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

REAGENTŲ INSTRUKCIJA NR. 3

Xpert® Flu/RSV XC

For *In Vitro* Diagnostic Use Only.

1 Proprietary Name

Xpert® Flu/RSV XC

2 Common or Usual Name

Xpert Flu/RSV XC Assay

3 Intended Use

The Cepheid Xpert Flu/RSV XC Assay, performed on the GeneXpert Instrument Systems, is an automated, multiplex real-time, reverse transcriptase polymerase chain reaction (RT-PCR) assay intended for the *in vitro* qualitative detection and differentiation of influenza A, influenza B, and respiratory syncytial virus (RSV) viral RNA. The Xpert Flu/RSV XC Assay uses nasopharyngeal swab and nasal aspirate/wash specimens collected from patients with signs and symptoms of respiratory infection. The Xpert Flu/RSV XC Assay is intended as an aid in the diagnosis of influenza and respiratory syncytial virus infections in conjunction with clinical and epidemiological risk factors.

Negative results do not preclude influenza virus or respiratory syncytial virus infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2013–2014 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

4 Summary and Explanation

Influenza, or the flu, is a contagious viral infection of the respiratory tract. Transmission of influenza is primarily airborne (*i.e.*, coughing or sneezing); the peak of transmission usually occurs in the winter months. Symptoms commonly include fever, chills, headache, muscle aches, malaise, cough, and sinus congestion. Gastrointestinal symptoms (i.e., nausea, vomiting, or diarrhea) may also occur, primarily in children, but are less common in adults. Symptoms generally appear within two days of exposure to an infected person. Pneumonia may develop as a complication of influenza infection, causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations.[1,2]

Influenza viruses are classified into types A, B, and C, the former two of which cause most human infections. Influenza A is the most common type of influenza virus in humans, and is generally responsible for seasonal flu epidemics and occasionally for pandemics. Influenza A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B virus are generally restricted to humans and are less frequent causes of epidemics. Influenza A viruses are further divided into subtypes on the basis of two surface proteins: hemagglutinin (H) and neuraminidase (N). Seasonal flu is normally caused by subtypes H1, H2, H3, and N1 and N2. In addition to seasonal flu, a novel H1N1 strain was identified in humans in the United States in early 2009.[3]

Respiratory syncytial virus (RSV), a member of the Paramyxoviridae family consisting of two strains (subgroups A and B), is also the cause of a contagious disease that afflicts primarily infants and the elderly who are immunocompromised, e.g., patients with chronic lung or heart disease or undergoing treatment for conditions that reduces the strength of their immune system.[3] The virus can live for hours on countertops and toys and causes both upper respiratory infections, such as tracheobronchitis and lower respiratory infections manifesting as bronchiolitis and pneumonia.[4] By the age of two, most children have already been infected by RSV, but because only weak immunity develops, both children and adults can become reinfected.[3] Symptoms usually appear four to six days after infection. The disease is typically self-limiting, lasting about one to two weeks in infants. In adults, the infection lasts about five days and presents with symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever. The RSV season overlaps with influenza season somewhat as infections begin to rise during the fall and continues through early spring.[3,4] RSV infections, however, also occur at other times of the year, although rarely.

REAGENTY INSTRUKCJA NR 4

GeneXpert® Carba-R

For Research Use Only. Not for Use in Diagnostic Procedures.

1. Proprietary Name

GeneXpert® Carba-R

2. Common or Usual Name

Carba-R Assay

3. Summary

The Cepheid GeneXpert® Carba-R Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* test designed for rapid detection and differentiation of the bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48}, and bla_{IMP-1} gene sequences associated with carbapenem-non-susceptibility in Gram-negative bacteria.

4. Principle of the Procedure

The GeneXpert (GX) Instrument Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time PCR assays. The systems consist of an instrument, personal computer, and preloaded software for performing tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the system, see the *GeneXpert Dx System Operator Manual* or the *GeneXpert Infinity System Operator Manual*.

The primers and probes in the GeneXpert Carba-R Assay detect proprietary sequences for the bla_{KPC} (KPC), bla_{NDM} (NDM), bla_{VIM} (VIM), bla_{OXA-48} (OXA-48), and bla_{IMP-1} (IMP-1) gene sequences associated with carbapenem-non-susceptibility in Gram-negative bacteria. Rectal swabs are collected using the Cepheid collection kit and transported to the testing laboratory. Material on the swab is eluted by breaking one swab into a vial containing Cepheid Sample Reagent followed by vortexing. The eluate is transferred to the sample chamber of the cartridge using the disposable transfer pipette provided in the GeneXpert Carba-R Assay kit. All reagents required for sample preparation and real time PCR analysis are preloaded in the cartridge. Bacterial cells in the eluate are mixed with the sample preparation control and treatment reagents, cells are captured on a filter and lysed by sonication. The released DNA is eluted, mixed with dry PCR reagent, and the solution is transferred to the reaction tube for real-time PCR and detection. Time to result is approximately 48 minutes.

5. Reagents and Instruments

5.1 Material Provided

The GeneXpert Carba-R Assay kit contains sufficient reagents to process 10 samples. The kit contains the following:

GeneXpert Carba-R Assay Cartridges with Integrated Reaction Tubes	**10**
• Bead 1, Bead 2, and Bead 3 (freeze-dried)	1 of each per cartridge
• Reagent 1	3 mL per cartridge
• Reagent 2:	
◦ Guanidinium chloride	2.5 mL per cartridge
GeneXpert Carba-R Assay Sample Reagent Vials	**10**
• Sample Reagent	**1 x 5.0 mL per vial**
Disposable (1.7 mL) Transfer Pipettes	**10**
CD	**1**