

## APPLICATION NOTE

## Drugs of Abuse Testing in Whole Blood

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### Introduction

Drugs of abuse are defined as substances that are consumed not for the treatment of disease, but for their mind-altering effects. This definition is not limited to illegal drugs; it also includes approved pharmaceuticals with abuse or habit-forming potential. Substances in this category include amphetamines, barbiturates, benzodiazepines, booster, cocaine, cannabinoids, opiates/opioids, Z-Drugs and other drugs of abuse (**The complete list of analytes can be found in table 2 on pages 2-6**).

Here we describe a simple and quick adaptation of the **MassTox**<sup>®</sup> Drugs of Abuse Testing in urine assay (order no. 96000) to enable the analysis of 108 substances in venous whole blood. The changes only affect sample preparation, while the analytical system can remain completely unchanged.

### Material and Methods

#### Reconstitution of calibrators/controls

Prior to sample preparation a 6PLUS1<sup>®</sup> Multilevel Calibrator Set (order no. 96039/0-6) and **MassCheck**<sup>®</sup> Control Level I-III (REF 0410, 0420, 0430) were reconstituted in combination with analyte-free lyophilised whole blood as follows:

- For calibrators 8.0 mL Reconstitution Buffer (order no. 96023) was pipetted into lyophilised analyte-free whole blood and exactly 1.0 mL of the reconstituted analyte-free whole blood was added into each vial of the calibrator and reconstituted it for 10 to 15 min at +20 to +25 °C swirling repeatedly.
- For each control level 2.5 mL Reconstitution Buffer (order no. 96024) was pipetted into lyophilised analyte-free whole blood and exactly 1.0 mL of the reconstituted analyte-free whole blood was added into each vial of the control level and reconstituted it for 10 to 15 min at +20 to +25 °C swirling repeatedly.

Dilution of Calibrator Level 1 with analyte-free whole blood may be required in order to meet legal requirements for blood analysis.

#### Sample Preparation

The sample preparation procedure was performed in accordance with the **MassTox**<sup>®</sup> Drugs of Abuse protocol for urine, omitting the hydrolysis step (hydrolysis is not required for whole blood samples). To compensate for the volume, 40 µL of Dilution Buffer (order no. 96010) was added instead. (See the following page)

- 50 µL of sample/calibrator/control was placed into a 1.5 mL reaction vial, adding 10 µL of the reconstituted Internal Standard Mix (order no. 96046) and 40 µL Dilution Buffer (order no. 96010), then mixing it for 2 s (vortex)
- 100 µL Precipitation Reagent (order no. 96005) was then added, mixing it for 2 s (vortex) and centrifuged it for 5 min at 15000 x g
- 150 µL Dilution Buffer (order no. 96010) was pipetted into an autosampler vial and 100 µL of the sample supernatant was added
- The closed autosampler vial was mixed for 2 s (vortex) prior to injection of the prepared sample into the LC-MS/MS system

*This workflow describes a use that is not covered by the intended purpose of the product. The procedure has not been validated by the manufacturer and is not intended for diagnostic use. All responsibility for performance, suitability and regulatory compliance lies with the user.*

### LC-MS/MS

For the LC-MS/MS method und analytical column, there are no changes to the original method required. Chromatographic separation was performed on an Analytical Column (order no. 96100) at a column temperature of 30 °C. The analytes are separated with a gradient profile at a flow rate of 0.4 mL/min using Mobile Phase A (order no. 96001) and Mobile Phase B (order no. 96002). See Table 1.

Time	Mobile Phase A	Mobile Phase B	Flow rate
0 min	100%	0%	0.4 mL/min
0.20 min	100%	0%	0.4 mL/min
10.20 min	0%	100%	0.4 mL/min
12.00 min	0%	100%	0.4 mL/min
12.01 min	100%	0%	0.4 mL/min
12.50 min	100%	0%	0.4 mL/min

Table 1: Gradient profile

Electrospray ionisation was used and the multiple reaction monitoring (MRM) transitions used for quantification are listed in Table 2 below and on the following pages.

Analyte	Internal Standard	MRM 1	MRM 2
<b>Amphetamines</b>			
Amphetamine (TQS)	Amphetamine ISTD 142 → 93	136 → 91	136 → 119
Amphetamine	Amphetamine ISTD 142 → 93	137 → 92	137 → 120
BDB	BDB ISTD 196 → 149	194 → 147	194 → 177
Butylone	Butylone ISTD 225 → 177	223 → 175	223 → 130
2C-B	2C-B ISTD 266 → 249	261 → 244	260 → 228
2C-I	2C-I ISTD 314 → 297"	309 → 292	309 → 277
Cathinone	Cathinone ISTD 155 → 110	150 → 105	150 → 117
MBDB	MBDB ISTD 213 → 136	209 → 136	209 → 178
MDA	MDA ISTD 185 → 138	180 → 133	181 → 164
MDEA	MDEA ISTD 213 → 163	209 → 164	209 → 106

Table 2: Used MRM transitions

Analyte	Internal Standard	MRM 1	MRM 2
MDMA	MDMA ISTD 197 → 163	195 → 164	195 → 106
MDPV	MDPV ISTD 284 → 134	277 → 127	277 → 176
Mephedrone	Mephedrone ISTD 181 → 163	179 → 161	179 → 145
Mephedrone (TQS)	Mephedrone ISTD 181 → 163	178 → 160	178 → 144
Methamphetamine	Methamphetamine ISTD 155 → 92	151 → 92	151 → 120
Methaqualone	Methaqualone ISTD 258 → 139	252 → 133	252 → 66
Methylone	Methylone ISTD 211 → 163	209 → 161	209 → 133
Methylphenidate	Methylphenidate ISTD 244 → 93	235 → 85	234 → 56
PMA	MDA ISTD 185 → 138	167 → 122	167 → 150
PMA (TQS)	MDA ISTD 185 → 138	166 → 121	166 → 149
Ritalinic acid	Ritalinic acid ISTD 224 → 88	221 → 85	220 → 56
<b>Benzodiazepines</b>			
Alprazolam	Alprazolam ISTD 316 → 288	310 → 282	310 → 206
7-Aminoclonazepam	7-Aminoclonazepam ISTD 290 → 226	286 → 222	286 → 121
7-Aminoflunitrazepam	7-Aminoflunitrazepam ISTD 287 → 138	285 → 136	284 → 227
7-Aminonitrazepam	7-Aminonitrazepam ISTD 257 → 121	253 → 122	252 → 146
Bromazepam	Bromazepam ISTD 320 → 213	316 → 209	316 → 182
Brotizolam	Diazepam ISTD 290 → 154	393 → 282	394 → 315
Chlordiazepoxide	Chlordiazepoxide ISTD 305 → 286	301 → 283	300 → 247
Clobazam	Clobazam ISTD 309 → 267	302 → 260	301 → 224
Clonazepam	Clonazepam ISTD 320 → 274	316 → 270	316 → 214
Demoxepam	Demoxepam ISTD 292 → 180	287 → 180	287 → 269
Desalkylflurazepam	Desalkylflurazepam ISTD 293 → 140	289 → 140	289 → 226
Desmethylflunitrazepam	Desmethylflunitrazepam ISTD 304 → 258	300 → 254	300 → 198
Diazepam	Diazepam ISTD 290 → 154	286 → 155	286 → 194
Estazolam	Estazolam ISTD 300 → 272	296 → 268	296 → 206
Flunitrazepam	Flunitrazepam ISTD 317 → 271	315 → 269	314 → 239
Flurazepam	Flurazepam ISTD 398 → 315	389 → 315	388 → 288
α-Hydroxyalprazolam	α-Hydroxyalprazolam ISTD 330 → 302	325 → 297	325 → 216
α-Hydroxymidazolam	α-Hydroxymidazolam ISTD 346 → 328	342 → 203	342 → 168
α-Hydroxytriazolam	α-Hydroxytriazolam ISTD 365 → 337	359 → 331	359 → 176
3-Hydroxybromazepam	3-Hydroxybromazepam ISTD 336 → 307	332 → 303	332 → 315
Lorazepam	Lorazepam ISTD 325 → 233	321 → 229	321 → 303
Lormetazepam	Lormetazepam ISTD 342 → 296	336 → 290	335 → 177
Medazepam	Medazepam ISTD 275 → 246	271 → 242	272 → 208

Table 2.1: Used MRM transitions

Analyte	Internal Standard	MRM 1	MRM 2
Midazolam	Midazolam ISTD 332 → 297	327 → 292	326 → 249
Nitrazepam	Nitrazepam ISTD 287 → 185	282 → 236	282 → 180
Norclobazam	Norclobazam ISTD 293 → 251	287 → 245	287 → 210
Nordiazepam	Nordiazepam ISTD 276 → 140	271 → 140	271 → 165
Oxazepam	Oxazepam ISTD 292 → 163	287 → 163	287 → 104
Prazepam	Prazepam ISTD 330 → 276	326 → 272	326 → 140
Temazepam	Temazepam ISTD 308 → 262	302 → 256	301 → 177
Triazolam	Triazolam ISTD 349 → 312	343 → 308	343 → 315
<b>Boosters</b>			
Gabapentin	Gabapentin ISTD 176 → 139	172 → 137	172 → 154
Pregabalin	Pregabalin ISTD 164 → 57	160 → 55	160 → 83
Promethazine	Promethazine ISTD 291 → 92	286 → 87	286 → 199
Quetiapine	Quetiapine ISTD 388 → 255	385 → 254	385 → 222
<b>Cocaine</b>			
Benzoylcegonine	Benzoylcegonine ISTD 293 → 171	291 → 169	290 → 82
Cocaethylene	Cocaethylene ISTD 321 → 199	319 → 197	318 → 82
Cocaine	Cocaine ISTD 307 → 185	305 → 183	304 → 77
Norcocaine	Norcocaine ISTD 293 → 171	291 → 169	290 → 105
<b>Cannabinoids</b>			
11-Nor-9-carboxy- $\Delta$ 9-THC	11-Nor-9-carboxy- $\Delta$ 9-THC ISTD 348 → 302	345 → 299	345 → 193
<b>Opiates/Opioids</b>			
Acetylcodeine	Acetylcodeine ISTD 345 → 225	342 → 225	342 → 165
Buprenorphine	Buprenorphine ISTD 472 → 400	468 → 396	468 → 414
Codeine	Codeine ISTD 306 → 218	300 → 215	300 → 165
Dihydrocodeine	Dihydrocodeine ISTD 308 → 202	302 → 199	302 → 128
EDDP	EDDP ISTD 281 → 234	279 → 234	279 → 250
Fentanyl	Fentanyl ISTD 342 → 188	337 → 188	337 → 105
Hydrocodone	Hydrocodone ISTD 306 → 202	300 → 199	300 → 171
Hydromorphone	Hydromorphone ISTD 289 → 185	286 → 185	286 → 157
Meconin	Meconin ISTD 198 → 105	195 → 105	195 → 77
Meperidine (Pethidine)	Meperidine ISTD 252 → 178	248 → 174	248 → 70
Methadone	Methadone ISTD 319 → 268	311 → 265	311 → 106
6-Monoacetylmorphine	6-Monoacetylmorphine ISTD 334 → 165	328 → 165	328 → 211
Morphine	Morphine ISTD 289 → 165	286 → 165	286 → 201
Naloxone	Naloxone ISTD 333 → 315	328 → 310	328 → 212

Table 2.2: Used MRM transitions

Analyte	Internal Standard	MRM 1	MRM 2
Naltrexone	Naltrexone ISTD 345 → 270	342 → 270	342 → 267
Norbuprenorphine	Norbuprenorphine ISTD 417 → 83	414 → 83	414 → 187
Norcodeine	Norcodeine ISTD 289 → 152	286 → 165	286 → 152
Norfentanyl	Norfentanyl ISTD 238 → 84	233 → 84	233 → 55
Normeperidine (Norpethidine)	Normeperidine ISTD 238 → 164	235 → 161	234 → 188
Nortapentadol	Tapentadol ISTD 225 → 107	209 → 108	208 → 121
Nortapentadol (TQS)	Tapentadol ISTD 225 → 107	209 → 122	–
Nortilidine	Nortilidine ISTD 263 → 155	261 → 155	261 → 230
O-Desmethyltramadol	O-Desmethyltramadol ISTD 256 → 64	250 → 58	250 → 42
O-Desmethyltramadol (TQS)	O-Desmethyltramadol ISTD 256 → 64	251 → 59	–
Oxycodone	Oxycodone ISTD 322 → 304	316 → 298	316 → 241
Oxymorphone	Oxymorphone ISTD 305 → 230	302 → 227	302 → 284
Papaverine	Papaverine ISTD 343 → 205	341 → 203	340 → 296
Papaverine (TQS)	Papaverine ISTD 343 → 205	341 → 297	–
Propoxyphene	Propoxyphene ISTD 345 → 58	341 → 58	341 → 267
Sufentanil	Sufentanil ISTD 392 → 238	388 → 238	388 → 112
Tapentadol	Tapentadol ISTD 225 → 107	223 → 108	222 → 121
Thebaine	Thebaine ISTD 315 → 61	312 → 58	312 → 251
Tilidine	Tilidine ISTD 280 → 155	275 → 155	275 → 78
Tramadol	Tramadol ISTD 267 → 58	265 → 58	264 → 42
<b>Z Drugs</b>			
Zaleplon	Zaleplon ISTD 311 → 237	307 → 237	307 → 234
Zolpidem	Zolpidem ISTD 314 → 235	309 → 235	309 → 264
Zopiclone	Zopiclone ISTD 393 → 245	390 → 246	390 → 218
<b>Others</b>			
Ketamine	Ketamine ISTD 242 → 129	239 → 126	238 → 220
LSD	LSD ISTD 327 → 226	324 → 223	324 → 208
Mescaline	Mescaline ISTD 221 → 171	212 → 165	212 → 180
Norketamine	Norketamine ISTD 228 → 129	225 → 126	225 → 208
2-Oxo-3-hydroxy-LSD	Ritalinic acid ISTD 224 → 88	356 → 222	356 → 166
PCP	PCP ISTD 249 → 86	244 → 86	244 → 91
<b>Barbiturates</b>			
Allobarbital	Phenobarbital ISTD 236 → 42	207 → 42	207 → 85
Amobarbital	Pentobarbital ISTD 230 → 42	225 → 42	225 → 182
Barbital	Phenobarbital ISTD 236 → 42	183 → 42	183 → 140

Table 2.3: Used MRM transitions

Analyte	Internal Standard	MRM 1	MRM 2
Butalbital	Butalbital ISTD 228 → 42	223 → 42	223 → 180
Hexobarbital	Secobarbital ISTD 242 → 42	235 → 42	–
Pentobarbital	Pentobarbital ISTD 230 → 42	225 → 42	225 → 182
Phenobarbital	Phenobarbital ISTD 236 → 42	231 → 42	231 → 188
Secbutabarbital	Butalbital ISTD 228 → 42	211 → 42	211 → 168
Secobarbital	Secobarbital ISTD 242 → 42	237 → 42	237 → 194
Thiopental	Secobarbital ISTD 242 → 42	241 → 58	241 → 101

Table 2.4: Used MRM transitions

### Quantification

For calibration and quality control, the Urine Calibration Set 96040 and **MassCheck**® Control Level I-III 0410, 0420 and 0430 were used, with following modification: Instead of the contained lyophilised urine matrix, we used lyophilised whole blood.

Calibration curves were constructed by calculating the analyte to internal standard (ISTD) peak area ratio on the y axis against calibrator concentrations on the x axis. Then a calibration curve was plotted using linear regression and  $1/x^2$  weighting.

### Results

The run time is 12.5 min (see chromatograms in fig. 1 + 2). The calibration range covers the forensic and clinical relevant concentrations (table 3). One limitation of the method is that the determination of THC and 11-OH-THC is not possible, as these analytes were not included in the calibrators used.

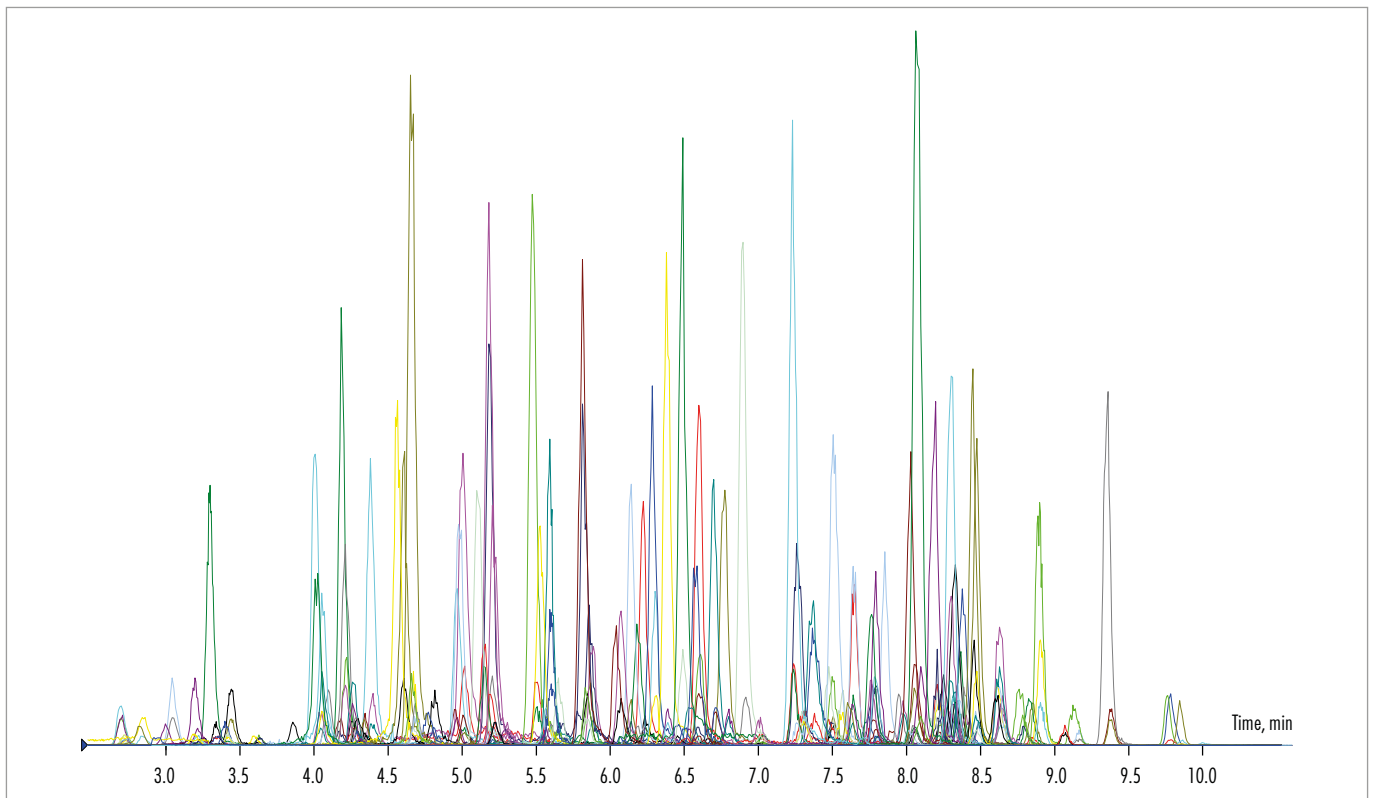


Fig. 1: ESI positive

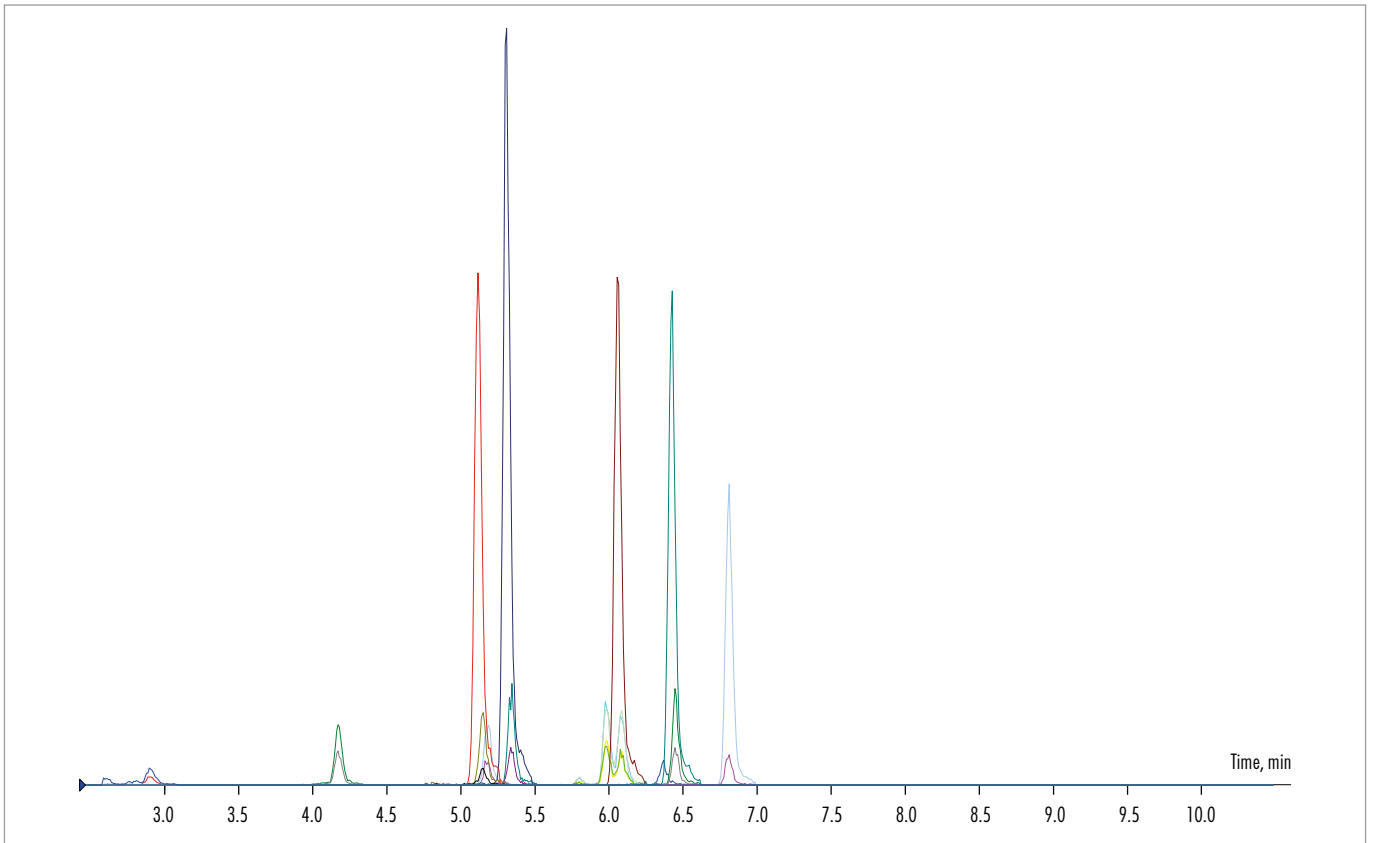


Fig. 2: ESI negative

In the following table 3 results of Arvecon proficiency scheme are presented. The scheme is passed for all analytes. The unit is µg/L

Analyte	Sample	Target value	Evaluation limits	Result	Z-score
Dihydrocodeine	A	120,0	66,0-174,0	98,3	-0,80
Amphetamine	A	75,5	39,7-111,3	72,1	-0,18
MDMA	A	66,8	34,6-99,0	63,3	-0,21
MDEA	A	64,5	33,3-95,7	65,9	0,08
MDA	A	57,6	29,2-86,0	52,5	-0,35
Methamphetamine	A	122,0	68,0-176,0	121,0	-0,03
Cocaine	B	30,0	13,6-46,4	29,7	-0,03
Benzoylcegonine	B	105,0	57,0-153,0	100,0	-0,20
Ketamine	B	633,0	415,0-851,0	610,0	-0,21
Norketamine	B	557,0	361,0-753,0	509,0	-0,48
Methadone	B	85,3	45,7-124,9	90,8	0,27
EDDP	B	58,5	29,7-87,3	53,7	-0,33

Table 3

The application of this method has already been tested with several hundred whole blood samples. No difficulties or equipment problems such as contamination were observed. Analysis of whole blood samples can also be performed in conjunction with urine samples using the original assay.

### Conclusion

This application note describes a protocol for the determination of drugs of abuse in whole blood using a slightly modified version of the Chromsystems kit 96000 **MassTox**<sup>®</sup> Drugs of Abuse in Urine, while retaining the components of the original method.

Furthermore, the kit has also been tested using other postmortem matrices such as vitreous humor and different organ blood, yielding promising results.

**Please note:** This workflow describes a use that is not covered by the intended purpose of the product. The procedure has not been validated by the manufacturer and is not intended for diagnostic use. All responsibility for performance, suitability and regulatory compliance lies with the user.

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