

\$GYDQFHV LQ 5RERWLFV *URVFKO HW DO \$XWRP DWL 5RERW \$

5HVHDFK \$UWLFOH

20,&6 ,QWH

" -JRVJE)BOEMJOH 3PCPU GPS 3PCVTU BOE 3F
4UBOEBSE BOE 2VBMJUZ \$POUSPM 4BNQMFT JC

0LFKDHO *URVFKO HW DO 7KLV LV DQ RSHQ DFFHVV DUWLFOH GLVWULEXWHG XQGHU WKH
&HOHULRQ ,QF 6ZLW]HUODQG \$* \$OOPHQGVWUDVHV)HKUDOWRUI 6ZLW]HUODQG
)RUQD[7HFKQRORJLHV *PE+ XQG &R .* ,P 6RQQHQODQG 1HXVWDGW :LHG *HUPDQ\
&RUUHVSRRGLQJ 0DFKDFURVFKO HOHULRQ]HUODQG \$OOPHQGVWUDVHV 6ZLW]HUODQG 7HO
PLFKDHO JURHVFKO#FHOHULRQ FRP
5HFHLYHG 1QDWH \$FFHSWHG- DQWH 3XEOLVKHG DQWH
&RSULJKW 0LFKDHO *URVFKO HW DO 7KLV LV DQ RSHQ DFFHVV DUWLFOH GLVWULEXWHG XQGHU WKH
XQUHVWULFWHG XVH GLVWULEXWLRQ DQG UHSURGXFWLRQ LQ DQ\ PHGLXP SURYLGHG WKH RULJLQDO D

\$EVWUDFW
:H GHVFULEH D XQLTXH OLTXLG KDQGOLQJ SODWIRUP EDVHG RQ D 7HFDQ (92 V
RI DQDO\WLF FDOLEUDWRUV DQG TXDOLW\ FRQWURO VDP SOHV DFFRUGLQJ WR WK
*/3 7KH SODWIRUP XWLOL]HV D FRPELQDWLRQ RI RII WKH VKHOI VRIWZDUH 7HF
63..(7KH V\WHP FRQLQFHV ZLWK UREXVW DQG UHSURGXFWLEO\ TXDOLW\ DQG
VHFUXLW\ UHTXLUHPHQWV DV SHU)\$ &)5SDUW ZHUH FRQVLGHUH G ZKHQ SURJL

.HIZRUGREXVW %LRDQDO\VLV /LTXLG SODWIRUP FRQWURO (PHQW \$ IXOO FRPS
DSSOLHG > @ EHIRUH VHWWLQJ WKH V\WHP

,QWURGXFWRQ

H SUHSDUDWLRQ RI DQDO\WLFDO VWDQGDUGV FDOLEUDWRUV DQG TXDOLW\
FRQWURO VDP SOHV 4&V LQ D */3 ODERUDWRU\ LV RQH RI WKH NH\ FKDOOHQJHV
GXULQJ WKH FRQGXFW RI SUHSDUDWLRQV FOLQLFDO VWXGLHV
RI */3 GHPDQG WKH LQGHSHQG HQW SUHSDUDWLRQ RI FDOLEUDWRUV DQG 4&V
ZKLFK EHLQJ PHDVXUH G VLPXOWDQHRXVO\ VKDOO PDWFK HDFK RWKHU ZLWKLQ
VWUHQF\ QHGLWHUHV SUHSDUDWLRQV PD\ QHHG WR EH
UHSHDWHG LI WKH VWXG\ UXQV RYHU ORQJHU WLPH RU DQ XQH[SHFWHG QXPEHU
RI UHSHWLWLRQV OHDG WR IXUWKHU GHPDQG RI VWDQGDUG DQG 4& PDWHULDO

\$ PDQXDO DSSURDFK LV WLPH DQG ODERU LQWHQVLYH 0RUHRYHU
PLVPDWFKHV EHWZHHQ FDOLEUDWRUV DQG 4&V WHQG WR KDSSHQ ZLWK
FRQVHTXHQWO\ UHSHDWHG DSSURDFK DLRIWJKHU
FRQVXPSWLRQ RI YDOXDEOH UHIHUHQFH XEVWDQFH XVHG VXFK DV VWRFN
PDWHULDO DQG EODQN PDWUL[H J DQDO\W IUHH VHXP DQG D ORQJHU OHDG
LQ XQWLO FDOLEUDWRUV DQG 4&V DUH DYDLODEOH IRU VDP SOHV PHDVXUHPHQW
)LJXUH 1 PLQL GHN OD\RXW IRU

\$ URERWLF DSSURDFK LQ FRQWUDV FRXOGE UDWRUWH GHFN &FRQVWDQW DQG UREXVW
GD\ E\ GD\ RU EXON SUHSDUDWLRQV RI FDOLEUDWRUV GLEWLG DQEN\DIRKHORSXUF
WR RYHUFRPH ZDVWH R\SDSHU GHVFDQVH D\XO Q\WR EH ORFDWHG RQ RQH UDF
OLTXLG KDQGOLQJ SODWIRUP DOORZLQJ FROLDQDWRU DQW 4&V SCSUHSUWLRQV IRO
IXOO\ DXWRPDWHG SURGXFWLRQ RI FDOLEUDWRUV GLEWLG W\WRQV\JEQQ DEHXVGRH
PDGHVZDSSOLFDFWLRQ EHLQJ LQ IXOO FRPSOLQFH ZLWK &)5 SDUW

6\WVHP 'HVFULSWLRQ

H URERWLF SODWIRUP FRQVLVWV RIGVFRVTHFRQVWHV DQW (92HG GRUHDQV
HTXLSSHG ZLWK DQ FKDQQHO OLTXLG KDQGOLQJ DQW PHV DQW EDUFRHDXU
VFDQQHU 3RV,' WZR RQ GHFN VKDNHUV FDQG GLYHUVH FDUULHUV IRU WXEHV
DQG WURXJKV 7HFDQ *HPLQL VRZVDFKRYHO DV WKH URERWLF WKH 63 (FXVWRZ
DGPLQLVWHULQJ WKH GHFN ODEZDUH DQG *HPLQLG LQGLYHUVH DQW WKH 63 (FXVWRZ
PDGH 63..()RUQD[DGPLQLVWHULQJ WKH GHFN ODEZDUH DQG *HPLQLG LQGLYHUVH DQW WKH 63 (FXVWRZ
GDWDEDVH DQG GULYLQJ WKH GHFN ODEZDUH DQG *HPLQLG LQGLYHUVH DQW WKH 63 (FXVWRZ
GDWDEDVH VZDSSOLFDFNDJH UXQV RQ :LQGRZY DQW DQW PHWKRGV IURP RV
LQVWDOOHG RQ PXOWLSOH 3&V WR DOORZ IRU VHSUDWLRQ LQ D GHYHORSHPHQW

&LWDWLEBQVFKO 0 ODUNXV \$ /H\HUV 6 6FKLEOL 5 =HOSJ HILT KLGLHDVGHOL QD QRECDULR U5 5REXV
3UHSUDWLRQ RI 6WDQGDUG DQG 4XDOLW\G&R\SPERRO \$ODPRD HV LQ %LRDQDO\VLV

3DJH RI

LQIRUPDWLRQ LV VWRUHG LQ D VTOLWH GDWDEDVH ZKHUH LW LV UHWULHYHG E
63..(H[H

H *HPLQVRVZDZHFDQ FRQWUROV WKH
DQG WKH SHULSKHUDO HTXLSPHQW EDV
W&DWHUHQVWZWHQ VRXUFH DQG GHVW
DQG ORZ YROXPHV LQFOXGLQJ WZR VKI
(LJKW FDUULHUV DUH UHVHUYHG IRU
SUHSDUHG OLTXLGV LQWR VPDOO FURW
H V\WHP FKHFNV EHIRUH GLVSHQVLQ
GHVWLQDWLRQ WXEHV KDYH WKH FRUHH
LVDRYLGV DFFLGHQWDO PL[LQJ RI LQF
VHULHV DQG ODWHU XQSODVVDQW VXSULYHV
VKRZV WKH DQDO\WLF UHVSQVH RQ FDOFXODWLRQ

Line	Final Solution ID	Final Solution Concentration (ug/ml)	Spiking Solution ID	Spiking Solution Concentration (ug/ml)	Final Volume according to Method (ml)	Solvent
1	WS1 STD	0.1	Stock 1	1.0	2.0	Methanol
2	WS1 STD	0.1	Stock 2	2.0	2.0	Methanol
3	WS1 STD	0.1	Stock 3	4.0	2.0	Methanol
4	WS2 STD	0.001	WS1 STD	0.1	2.0	Plasma

Final Solution ID	Final Solution Concentration (ug/ml)	Spiking Solution ID	Final Volume according to Method (ml)	Solvent volume (ml)	Source volume (ml)	Solvent
1	WS1 STD	WS1 STD	1000.0	2.0	1.4	Methanol
2	WS1 STD	WS1 STD	1000.0	0	0.2	Methanol
3	WS1 STD	WS1 STD	1000.0	0	0	Methanol
4	WS1 STD	WS1 STD	1000.0	2.0	14.2	Methanol
5	WS2 STD	WS1 STD	1000.0	0	0	Methanol
6	WS2 STD	WS1 STD	1000.0	0	0.19	Methanol
7	WS2 STD	WS1 STD	1000.0	0	0.19	Methanol
8	WS2 STD	WS1 STD	1000.0	2.0	1.82	Methanol
9	WS2 STD	WS1 STD	1000.0	0	0.06	Methanol
10	WS2 STD	WS1 STD	1000.0	0	0.06	Methanol

6XEVHTXHQWO\ D fVHWg LV FUHDWHG D
SRVLWLRQV RQ WKH GHFN OD\RXW DQG W
VROYHQW RI LQWHUHV %\ VDYLQJ WKH
DFFHVLEOH WR WKH HQG XVHU WR H[HF
GHVLUH VROXWLRQV RQ PMKH6LQFH FDO
HPEHGGHG LQWR WKH 63..(W&DWHUHQVWZWHQ
DQG GRZQ RQ GHVWLQDWLRQ DQDO\WLF
UHTXLUH YROXPHV RI VWRUHG VROXWLRQ
UHFRLJQL]HV ZKHWHU WKH YROXPH WR E
YROXPH WLSV DQG ZLOO DGMXVW WKH DVS
DFFRUGLQJO\

H PDLQ IUDPH RI WKH 63..(DSSURDUJ
FROXPQV ,Q D f&UHDWH OHWKRGg PRGH
RQ ,V DQG FRQFHQWUDWLRQV RI WKH
WKH DQDO\WLF WR EH SUHSDUHG 6LQFH
VDPH LQ PRV FDUULHV FDQRW EH SUHSDUHG
FRQFHQWUDWLG VWRUHG VROXWLRQ LQWHU
DUH UHTXLUH)RU PXOWL DQDO\WLF
SUHSDUDWLRQ RI ZRUNLQJ VROXWLRQV
ZLWK WKH GLOXHQW YROXPH EHLQJ FRUHH

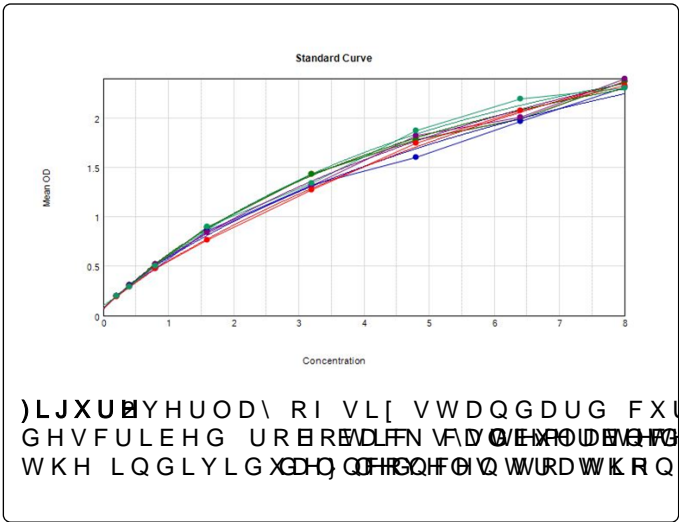
&LWDWLEBRVFKO 0 ODUNXV \$ /H\HUV 6 6FKLEOL 5 =HOSJ HLT KLGLHDVGHOLQJDQSEEDU LRU55REXV
3UHSUDWLRQ RI 6WDQGDUG DQG 4XDOLW\G&RSHQVRRR \$DQPH HV LQ %LRDQDO\VLV

3DJH RI

5HVXOWV

&RQFOXVLRQ

)RU ELRVLPLODUV DV DQ HPHUJLQJ PDUNHDXWRP DLWHGV RRAZDUNHVDGGEW B &MVHVVDV
LQGHSHQGHWQW\ SUHSDUHG VWDQGDUGWR XEJY HDV YDQXJDE B WURRPOVMR RFNPSD RYHQLQJ
IURP WKH RULJLQDWRU GUXH DQSDWLKHWELDVERPDDVU DQXJ TXDOLW\ FRPHWURRQGV DFP
YDULDELOLWLHV IURP WKH SUHSDUDWLRQVDQDQDPEFK BQVSRVNGE OVR LQKRUG B,U(WR N
WKH UHJUHQH FH VXEVDQDFHV WKH RQDQDXWKYDQJWRER BWHDDVDQDFQRRUR GDV EBR VLPWQ
(/,6\$ PHWKRQ ZH XVHG RXU URERWLFXQSSXIRDFKD VQGV FSLQSDRISH WDKWVRQ ZLWK
VWDQGDUG FXUYHV YLD VHYHUDO GLORXWLRQ &HVVHULRQDQZLWURRQDQDWHNP VLKQ
DOWHUQDWLQJ RUGHU RQ WKH DVVD\ SODVGLDWRWRRWRH &VHGLEH QDRELRQLLAWO IDT
> @H VWDQGDUG FXUYHV \LHOGHG IURPDSIOHV SURFENSHQGHEXW LQREVRWLRU WKH
SUHSDUDWLRQV ZHUH IRXQG WR EH ZHOULQJDFRKRUGDQGHQWRI SDIFKRLQKADQORV
RQ\ ZLWKLQ WKH SUHSDUDWLRQV IURP RQH UHJUHQH FH VXEVDQDFH EXW DOVR
ZKHQ FXUYHV ZHUH FRPSDUHG EHWZHHGRIHULHQDWRV DQG ELRVLPLODU)LJXUH
H EDFN FDQEXDQDFHGHQ WKH LQGLYLGXDO OHYHOV WR WKH
WDUJHW FRQFHQWUDWLRQ QHYHU H[FHHGHG &DQLEUDWRUV DQG 4&V ZKLFK
VKDOO QRQLH BQGH @ QHYHU H[FHHGHG *XLGDQFH FRPPQXWVQHWKRQ 9DOLGDWLRQ &(5 &90 SS



)'\$ (OHFWURQLF 5HFRUGV (OHFWURQLF 6LJQDWXUHV)LQD
&)5 3DUW *\$03 \$ 5LVN EDVHG&SSURDFKWRWRPSS
%DQ%DQDV %XVK (' +DPLWRQ 0 4&DQJF BWSROIWLFDQ ,QVWUXPHQWV I
3KDUPDFHXWLFRLHSSURDFK \$S36 3KDUP 6FL7HFK
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EHWZHHQ D ELRVLPLODU DQG D UHJUHQH FH ELRWKHUSSHXLW FRPPLWVHH UH
OLJDQG ELQGLQJ DVVD\ WR VXSSRUW SKDUPFRNLQHWLF DVVHVVPHQWV \$S36 -

)LJXUH YHUOD\ RI VL[VWDQGDUG FXUYHV VHSUDWHO\ SUHSDUHG E\ WKH
GHVFULEHG URREDFN V\QVRODQDFHGHQ
WKH LQGLYLGXDO OHYHOV WR WKH WURDWKRQ QHYHU H[FHHGV