IdentiClone® Dx

Instructions for Use

IdentiClone® Dx *IGH* Assay

For identification of clonal immunoglobulin heavy chain (*IGH*) gene rearrangements.

IVD For *in vitro* diagnostic use.

Catalog Number	Product(s)	UDI	Quantity	Storage Conditions
REF 91010101	IdentiClone Dx *IGH* Assay	00810022732502	33 Reactions	-30°C -15°C

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CE 2797 IVD

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1. Intended Use

The IdentiClone Dx *IGH* Assay ("Assay") is an *in vitro* diagnostic device intended for capillary electrophoresis based-detection of clonality in immunoglobulin heavy chain (*IGH*) gene rearrangements in peripheral blood specimens as an adjunctive method for the diagnosis of patients suspected to have a B-cell lymphoproliferative disease.

Positive results (*i.e.,* the detection of clonality) should not be the sole criterion for determining presence of disease. Negative results do not preclude lymphoproliferative disease. The use of additional laboratory testing (*e.g.,* white blood cell [WBC] counts, morphology, immunohistochemistry, detection of driver mutations, flow cytometry, etc.) and clinical presentation must be taken into consideration in the final diagnosis of lymphoproliferative disease.

This qualitative, non-automated Assay is for use on the ABI 3500xL Dx and ABI 3500xL Genetic Analyzers.

2. Indications / Contraindications

No contraindications are identified.

3. Glossary and Abbreviations

3.1. Glossary

Table 1. **Glossary of terms specific to the IdentiClone Dx *IGH* Assay**

Term	Definition
Amplicon	Multiplied copies of DNA fragment created via amplification (e.g., PCR).
Amplification	Making multiple copies of a gene or DNA sequence (e.g., PCR).
Artifact	A red peak detected by fragment analysis on the ABI 3500xL Dx Genetic Analyzer that is within 0.5 base pairs of up to top 5 highest blue or green peaks. If the artifact peak is at or greater than the associated blue or green peak, the associated master mix (MM) result will be considered invalid.
Clan	*IGH* gene rearrangements are identified by the IdentiClone Dx *IGH* Assay through the use of primers targeting the 7 IGHV subgroup genes, IGHV1 –7. The IGHV subgroup genes can be further categorized into IGHV gene clans (Clan I, II and III) based on their sequence similarity.[15] Clan I includes IGHV1, IGHV5 and IGHV7 subgroup genes, clan II includes IGHV2, IGHV4 and IGHV6 subgroup genes, and clan III includes IGHV3 subgroup gene.
Clonal	• A *Sample ID* result (overall call) in which Clonality is detected. • A *Sample Name* result (for a MM) in which a Significant Peak is detected in the valid size range.
Detection	Part of the Assay in which amplicons are separated by capillary electrophoresis and detected as peaks.
In Vitro	Occurring outside of a living organism.
Indeterminate	A sample result in which all 3 master mix (MM) results generated indeterminate results; or a sample MM result in which the presence or absence of clonality cannot be determined (i.e., ambiguous result)
Invalid	A sample result in which the 3 master mix results are Invalid; or a sample MM result which does not meet the validity criteria (see Table 8).
Lymphoproliferative disease	A disorder characterized by the abnormal proliferation of lymphocytes.
Master Mix Product	Amplicons generated from the amplification of 3 different MM: *IGH* Tube A (FR1) MM, *IGH* Tube B (FR2) MM, *IGH* Tube C (FR3) MM.
Negative Control	A buffer solution containing polyclonal DNA; this control is expected to generate a *Non-Clonal result with each master mix.*
Non-Clonal	A sample result in which clonality is not detected; or a sample MM result in which a significant peak is not detected within the valid size range.

Table 1. Glossary of terms specific to the IdentiClone Dx *IGH* Assay

Term	Definition
Platemap or Plate Map	Visual representation of a detection plate, imported to the ABI Genetic Analyzer. This file provides a 96-well plate layout containing associated run information, including *Sample ID*, *Sample Name*, *Sample Type*, and *Master Mix* for each well location.
Positive Control	A buffer solution containing DNA used to assess assay validity; this control is expected to generate a Clonal result with each master mix.
Sample ID	Unique identification associated with a patient sample. Each *Sample ID* is required to be tested with each master mix (N = 3) included with the Assay. Individual master mix and run-specific test results are identified with *Sample Name*. Each *Sample ID* will have at least 3 associated Sample Name results. See Figure 10 for an example.
Sample Name	Unique identification associated with master mix and run-specific test results associated with a *Sample ID* (Patient Sample). See Figure 10 for an example.
Saturation	Presence of peak with excessively high RFU (≥ 30,000)
Size Quality (SQ) Error	An ABI 3500xL or ABI 3500xL Dx Genetic Analyzer determined error in which the calculated similarity between the fragment pattern of the specific size standard dye and the observed distribution of size standard peaks in a sample did not pass the predetermined threshold.
Significant Peak	A dominant peak within a valid size range.

3.2. Abbreviations

Table 2. Abbreviation definitions

Acronym	Definition
bp	Base pair
CE	Capillary electrophoresis; an electrokinetic method used to separate amplicons by size.
CI	Confidence interval
DNA	Deoxyribonucleic acid
FNC	File Name Convention; a framework for naming files in a way that describes what they contain and how they relate to other files.
FSA	Fragment analysis data file created by the capillary electrophoresis instrument.
gDNA	Genomic DNA
IFU	Instructions for Use
IGH	Immunoglobulin heavy chain gene
***IGH* FR1/2/3**	The conserved frameworks 1, 2 and 3 if the *IGH* gene
IUO	Investigational Use Only
IVD	*In vitro* diagnostic
LL	Lower limit
LoB	Limit of blank
LoD	Limit of detection
MM	Master mix
N/A	Not applicable
NTC	No template control (water); this control is expected to generate no amplified peaks within the valid size range.
NPA	Negative percent agreement
OPA	Overall percent agreement
PB	Peripheral blood
PCR	Polymerase chain reaction

Table 2. Abbreviation definitions

Acronym	Definition
PPA	Positive percent agreement
PPB	Pooled peripheral blood
RPR	Relative Peak Ratio, the value used to determine clonality.
RUO	Research use only
SQ	Size Quality; a numerical presentation of the similarity between the fragment pattern for the size standard dye specified in the size standard definition and the actual distribution of size standard peaks in the sample.
XLSX	A file format compatible with Office 2010 versions and above. This file format can be used to create the plate record on the ABI 3500xL Dx and ABI 3500xL Genetic Analyzers.
XML	Extensible markup language file designed to store and transport data. This file format is used for the IdentiClone Dx *IGH* Assay Instrument Parameters, Results Group and File Name Convention for the ABI 3500xL Dx and ABI 3500xL Genetic Analyzers (included with REF 91010111).

4. Summary and Explanation of the Test

4.1. Background

It is estimated that over 900,000 new cases of B-cell lymphoproliferative diseases are diagnosed worldwide each year, considering the variety of diseases that fall in this category.[1,2,3] B-cell lymphoproliferative disorders, such as lymphomas and leukemias, often arise due to dysregulation of the normal processes of B-cell development, particularly in immunoglobulin heavy chain *(IGH)* gene rearrangements.

During ontogeny in B lymphocytes, the *IGH* gene undergoes a process called V(D)J recombination, where the variable (V_H), diversity (D_H), and joining (J_H) gene segments are randomly rearranged.[4,5] These rearranged gene segments increase genetic diversity, generating approximately 10^{12} unique DNA sequences, and enable the immune system to recognize a wide array of antigens.[4,5,6] However, in B-cell lymphoproliferative diseases, a single B-cell clone with a particular *IGH* rearrangement proliferates abnormally, leading to a population of B-cells with identical (or clonal) *IGH* gene rearrangements. Detection of these clonal expansions is a hallmark of B-cell malignancies.[4,5,6,7,8,9]

When polymerase chain reaction (PCR) is applied to these gene rearrangements, products unique in length and sequence are generated for each cell.[6,7,8,9] Thus, this methodology can be applied to identify lymphocyte populations derived from a single cell by identifying the unique V-J gene rearrangements present within the *IGH* locus.[6,8,9,10] The presence of clonal *IGH* rearrangements is used to confirm the diagnosis of B-cell lymphoproliferative diseases, distinguishing malignant from benign lymphoid proliferations.[11,12,13,14]

4.2. Summary

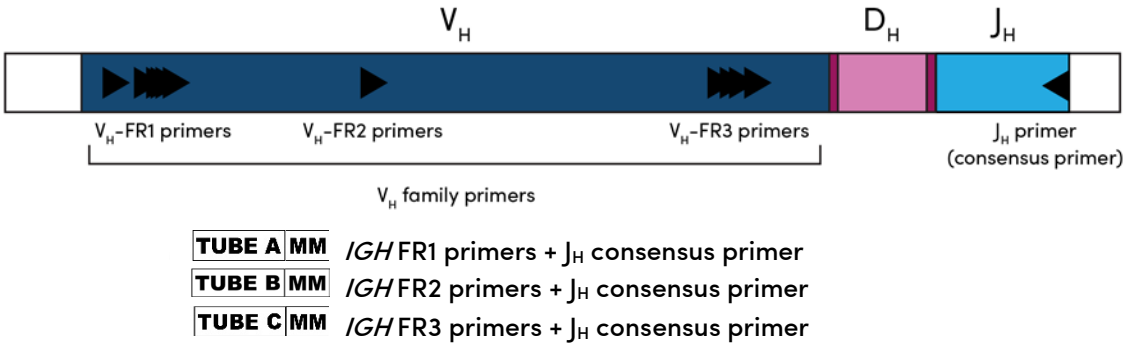
The IdentiClone Dx *IGH* Assay includes three PCR master mixes (MMs), a positive (*IGH* POS [+]) control, a negative (NEG [-]) control, a no template control (NTC), Taq DNA polymerase, and the IdentiClone Dx *IGH* Software Package. Assay master mixes employ multiple fluorescently-labeled consensus primers designed to target the conserved frameworks (FR1, 2, and 3) within the V_H and J_H regions of the *IGH* locus. Following amplification, fluorophore-labeled amplicons are fractionated by size, generating amplicon profiles with each PCR master mix. The resulting data is then uploaded to the IdentiClone Dx *IGH* Software for analysis to determine validity and clonality status. Valid results from each master mix are compiled to establish the clonality status for each specimen: Clonal, Non-Clonal, Invalid, or Indeterminate.

5. Principles of the Procedure

5.1. Polymerase Chain Reaction (PCR)

Because the variable region (V_H) is subject to somatic mutations, , it is difficult to employ a single set of PCR primers to target all the conserved regions flanking V_H-J_H rearrangements. For that reason, the IdentiClone Dx *IGH* Assay utilizes multiple consensus PCR primers that target three frameworks (FR1, FR2 and FR3) and the J_H region of the *IGH* gene. The consensus primers are conjugated to fluorescent dyes and contained in three individual master mixes: MMA, MMB and MMC. (Figure 1) These master mixes amplify the three framework (FR) and two complementarity-determining regions (CDRs) from genomic DNA (gDNA) isolated from peripheral blood specimen.

Figure 1. **The organization of a rearranged immunoglobulin heavy chain gene on chromosome 14. Black arrows represent the relative positions of primers that target the conserved framework (FR1/2/3) and the downstream consensus J_H gene segments. The amplicon products generated from each of these regions can be detected when fluorescent primer sets are used with capillary electrophoresis instruments that employ fluorescence detection.**



5.2. Fluorescence Detection

Following amplification, each PCR product is processed on an ABI 3500xL Dx or ABI 3500xL Genetic Analyzer instrument, in which fluorophore-labeled amplicons are fractionated by size . Assay-specific run parameters, such as instrument settings, results group and file naming convention, are included as XML files in the IdentiClone Dx *IGH* Software Package and must be configured prior performing the assay for the first time. Once configured, the amplicon profiles of each PCR master mix are collected as FSA file format and uploaded to the IdentiClone Dx *IGH* Software for interpretation.

5.3. Software Analysis

The IdentiClone Dx *IGH* Software is designed to complement the IdentiClone Dx *IGH* Assay and eliminate the subjectivity from electropherogram interpretation. The raw data files generated by the assay amplicons with fluorescence detection are analyzed for validity and clonality status while referencing a configured platemap for sample traceability.

Since this assay requires three master mixes to determine the clonality status, a naming hierarchy is used to correlate the master mix results, identified by a *Sample Name*, to the patient sample, which is identified by a *Sample ID* (see Figure 9). Each master mix test set, including samples, a positive control, a negative control, and a no template control, is considered a "run," can be configured individually using the Software Plate Setup function and loaded onto the same plate, containing additional runs. After detection is complete, the data files are uploaded to the Software, which proceeds with analysis, referencing the platemap to complete the intermediate analysis for that master mix. If runs for all three master mixes are determined to be valid, the data is processed to generate intermediate results, depicted by *Sample Names*, which are displayed for the user to choose and generate the clonality status for each *Sample ID*. For more details, please see the corresponding IdentiClone Dx *IGH* Software IFU.

5.4. End User and In-Use Environment

5.4.1. The Device is for professional use only in a clinical laboratory setting.
5.4.2. The Device is not intended for near-patient testing nor self-testing.
5.4.3. The Device is not a companion diagnostics assay.

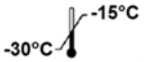
6. Reagents

6.1. Reagent Components

Table 3. Available Kits

Catalog Number	Description	Quantity
REF 91010101	IdentiClone Dx *IGH* Assay	33 Reactions

Table 4. Reagent Components (Components in Kit)

Reagent	Part Number	Reagent Components (active ingredients)	Unit Quantity	# of Units	Storage Temperature
Master Mixes	REF 21010191	**MMA** *IGH* FR1 Multiple oligonucleotides targeting the framework 1 region of the immunoglobulin heavy chain gene in a buffered salt solution.	1500 µL	1	
	REF 21010201	**MMB** *IGH* FR2 Multiple oligonucleotides targeting the framework 2 region of the immunoglobulin heavy chain gene in a buffered salt solution.	1500 µL	1	
	REF 21010211	**MMC** *IGH* FR3 Multiple oligonucleotides targeting the framework 3 region of the immunoglobulin heavy chain gene in a buffered salt solution.	1500 µL	1	
IGH Positive Control DNA	REF 40883460	**CONTROL +** *IGH* gDNA from cultured cells in buffered salt solution	100 µL	1	
IGH Negative Control DNA	REF 40920070	**CONTROL -** *IGH* gDNA from cultured cells in buffered salt solution	100 µL	1	
No Template Control (NTC)	REF 40930010	**CONTROL NTC** Water	100 µL	1	
FalconTaq® DNA Polymerase	REF 60970150	**TAQ** FalconTaq DNA Polymerase Enzyme	50 µL	1	
IdentiClone Dx *IGH* Software Package USB	REF 91010111	IdentiClone Dx *IGH* Software IdentiClone-Dx-*IGH*-Software-1.1.x.IVD.msi IdentiClone Dx *IGH* Assay Parameters IGH_IP.xml IGH_FNC.xml IGH_RG.xml	USB	1	

6.2. Reagent Storage and Handling

6.2.1. For any duration other than immediate use, **store assay kit at -30°C to -15°C**.

6.2.2. Upon opening the assay kit, visually inspect each reagent for damage and/or leaks.

6.2.3. Thaw and vortex all reagents and controls thoroughly prior to use to ensure that they are completely resuspended and homogenous.

IMPORTANT! Excessive vortexing may shear DNA and cause labeled primers to lose their fluorophores. Do NOT vortex the tube containing Taq DNA Polymerase.

6.2.3.1. Vortex volume at maximum speed for 5 to 15 seconds.

6.2.3.2. Centrifuge 2 to 5 seconds.

6.2.3.3. When stored and handled as directed, opened materials are stable up to 6 months, or 5 freeze-thaw cycles, or until the labeled expiration date. Do not use kits beyond their expiration date.

6.3. Warnings and Precautions

- **IVD** This product is for professional use only.
- Use this assay kit as a system. Do not substitute other manufacturer reagents. Dilution, reducing amplification reaction volumes, or other deviations to this protocol may affect the performance of this test and/or nullify any limited sublicense that comes with the purchase of this testing kit.
- Adherence to the protocol will assure optimal performance and reproducibility. Ensure the correct thermal cycler program is used, as other programs may provide inaccurate/faulty data, e.g., false positive and false negative results.
- Do not mix or combine reagents from kits with different lot numbers.
- Laboratory personnel must wear appropriate personal protective equipment and follow good laboratory practices and universal precautions when working with specimens. Always handle specimens in approved biological safety containment facilities and open only in certified biological safety cabinets.
- Due to the analytical sensitivity of this test, take extreme care to avoid contamination of reagents and/or amplification mixtures with samples, controls or amplified materials. Monitor all reagents for signs of contamination (*e.g.,* negative controls giving positive signals). Discard reagents suspected of contamination.
- To minimize contamination, wear clean gloves when handling samples and reagents and routinely clean work areas and pipettes prior to performing PCR.
- Autoclaving does not eliminate DNA contamination.
- Workflow in the PCR laboratory operates unidirectionally between separate work areas; begin with specimen preparation, move to master mix preparation, then to amplification, and finally to detection. Do not bring amplicons (i.e. PCR plate after amplification) into the areas designated for master mix or specimen preparation.
- Keep all pipettes, pipette tips, and any equipment used in a particular area dedicated to that area of the laboratory.
- Use sterile, disposable plastic ware whenever possible to avoid RNase, DNase, or cross-contamination.
- Once a POP-7 Polymer pouch has been brought to room temperature, examine inside the neck at the point of installation. Ensure the pouch is free of dried or crystallized polymer. Do not install the pouch on the ABI 3500xL Dx Genetic Analyzer instrument if crystallization is observed, as crystallization may impact the performance of the Assay and/or instrument. Contact Thermo Fisher Scientific customer support.

6.4. Reagents, Materials, and Equipment

Maintain all equipment according to the manufacturer instructions. Table 5 lists necessary reagents, materials, and equipment not provided in the kit.

Table 5. Reagents, Materials, and Equipment Required (not provided)

Reagent / Material / Equipment	Recommended Suppliers and Reagents / Materials / Equipment	Notes
8-cap Strips	N/A	RNase, DNase, DNA, PCR inhibitor free
96-well Septa	Thermo Fisher Scientific: • Septa for 3500xL Dx Genetic Analyzers, 96-well • Septa for 96-Well Plates, for 3500/SeqStudio™ Flex	N/A
Adhesive Plate Sealing Foil	N/A	RNase, DNase free
Anode Buffer Container	Thermo Fisher Scientific: • Anode Buffer Container for 3500 Dx/3500xL Genetic Analyzers • Anode Buffer Container (ABC), for 3500/SeqStudio Flex	RNase, DNase free
Calibrated Pipettes	N/A	Must be able to accurately measure volumes between 1 µL and 1000 µL
Capillary Array	Thermo Fisher Scientific: • 3500xL Dx Genetic Analyzer 24-Capillary Array, 50 cm • 3500xL Genetic Analyzer 24-Capillary Array, 50 cm	N/A
Capillary Electrophoresis Instrument	Thermo Fisher Scientific: • (EU) 3500xL Dx Genetic Analyzer with 3500 Dx Series Data Collection Software 3 IVD v3.0 or higher • (US) 3500xL Dx Genetic Analyzer with 3500 Dx Series Data Collection Software 3 IVD v3.2 or higher • 3500xL Genetic Analyzer with 3500 Series Data Collection Software v3.1 or higher	Ensure instrument is calibrated with DS-33 Matrix Standard Kit (Dye Set G5) with GeneScan 600 LIZ Size Standard v2.0
Cathode Buffer Container	Thermo Fisher Scientific: • Cathode Buffer Container for 3500 Dx/3500xL Genetic Analyzers • Cathode Buffer Container (CBC), for 3500/SeqStudio Flex	N/A
Cathode Buffer Septa	Thermo Fisher Scientific: • Septa Cathode Buffer Container for 3500 Dx/3500xL Dx Genetic Analyzers • Septa Cathode Buffer Container, for 3500 and SeqStudio Flex	N/A
Conditioning Reagent	Thermo Fisher Scientific: • Conditioning Reagent for 3500 Dx/3500xL Dx Genetic Analyzers • Conditioning Reagent, for 3500/SeqStudio Flex	N/A
Filter Barrier Pipette Tips	N/A	Sterile, RNase/DNase/Pyrogen-free
Hi-Di Formamide	Thermo Fisher Scientific: • Hi-Di™ Formamide, 3500 Dx Series • Hi-Di Formamide	N/A
Low-Bind Tubes	• N/A	For storing gDNA; RNase, DNase, DNA, PCR inhibitor free
LIZ Size Standards	Thermo Fisher Scientific: • GeneScan™ 600 LIZ® Size Standard v2.0 – Dx • GeneScan 600 LIZ dye Size Standard v2.0	N/A

Table 5. **Reagents, Materials, and Equipment Required (not provided)**

Reagent / Material / Equipment	Recommended Suppliers and Reagents / Materials / Equipment	Notes
Microcentrifuge	N/A	Sterile
Microvolume UV-Vis Spectrophotometer	N/A	Capable of measuring absorbance at 260 nm for nucleic acid concentration calculation
PCR Plates or Tubes	N/A	RNase, DNase, DNA, PCR inhibitor free
Plate Centrifuge	N/A	Centrifuge capable of 1000 RCF
POP-7 Polymer	Thermo Fisher Scientific: • POP-7™ (384) Performance Optimized Polymer 3500 Dx Series • POP-7 Polymer, for 3500/SeqStudio™ Flex	N/A
Retainer and Base Set	Thermo Fisher Scientific: • Retainer and Base Set (Standard) for 3500 Dx/3500xL Dx Genetic Analyzers, 96-well • Retainer & Base Set (Standard) for 3500/3500xL Genetic Analyzers, 96 well	N/A
Sterile Water	N/A	Sterile, DNase/RNase-free
Thermal Cycler	Thermo Fisher Scientific: • Veriti™ Dx	N/A
Tris-EDTA (TE) Buffer	N/A	Solution of 10 mM Tris-HCl, pH 8.0 and 1 mM EDTA Note: dilute 1:10 with water
Vortex Mixer	N/A	N/A

Contact manufacturer for item part numbers in your region.

7. Specimen Collection and Preparation

7.1. Precautions

Biological specimens from humans may contain potentially infectious materials. Handle all specimens in accordance with the OSHA Standard on Bloodborne Pathogens or Biosafety Level 2.

7.2. Interfering Substances

The following substances are known to interfere with PCR amplification, avoid if possible:

- Divalent cation chelators
- Low retention pipette tips
- EDTA (not significant at low concentrations)
- Heparin

7.3. Specimen Requirements and Stability

This Assay requires at least 0.5 mL of peripheral blood anti-coagulated with EDTA.

- Specimen can be stored at 2°C to 8°C up to 7 days prior to testing.
- Specimen may be shipped with a cold pack; it should never be frozen.

7.4. Sample Preparation

7.4.1. Extract the gDNA from specimens before 7 days from collection.
7.4.2. Quantify extracted gDNA using a microvolume UV-Vis spectrophotometer.
7.4.3. Prepare a dilution of gDNA in 50 ng/µL with 1/10th TE.

7.4.3.1. For amplification with each master mix, 5 µL of gDNA at a concentration of 50 ng/µL is required.

- Prepare at least 15 µL - 20 µL of diluted gDNA.

7.4.3.2. If the concentration of gDNA available is <50 ng/µL, re-extract the patient specimens.

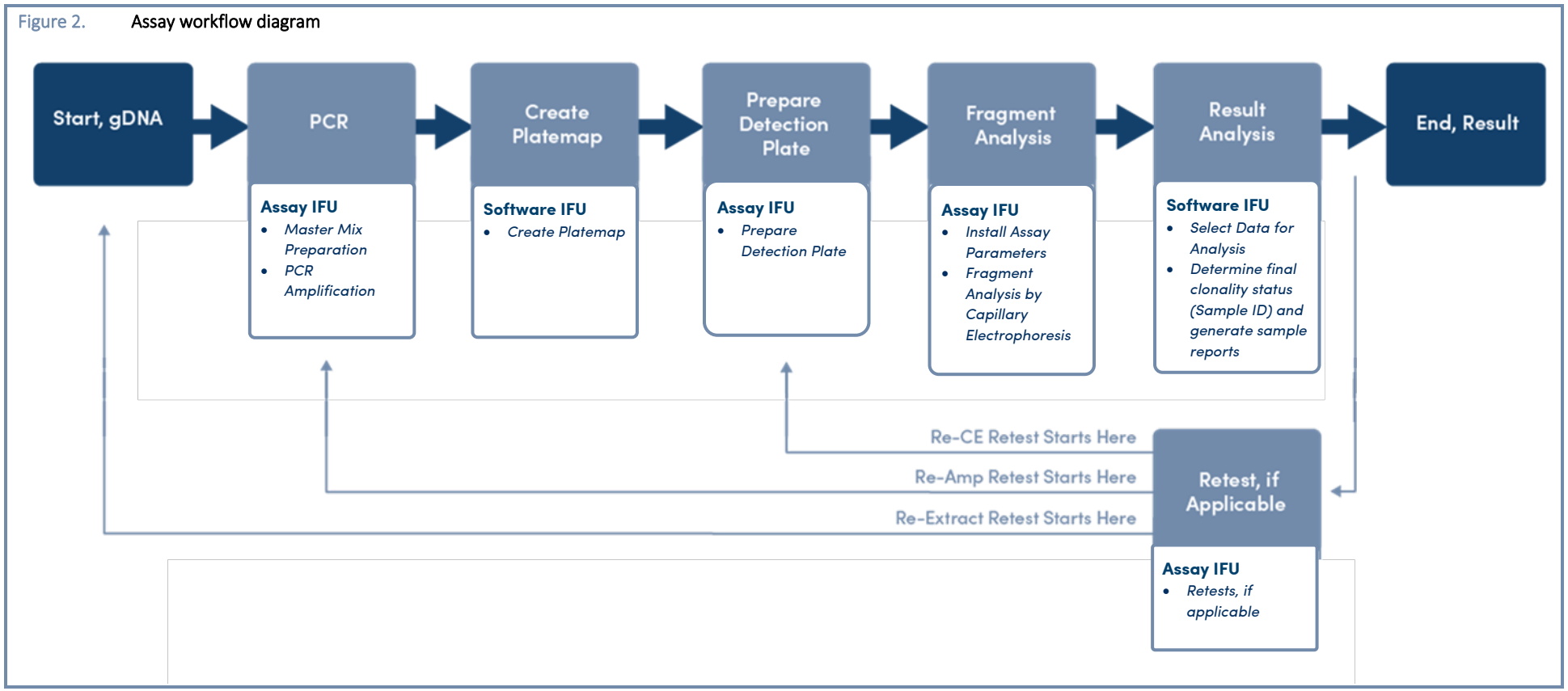
7.5. Sample Storage

7.5.1. Store gDNA at 2°C to 8°C up to 7 days before use or at -30°C to -15°C up to 3 years before use.
7.5.2. Store diluted gDNA samples in low-bind tubes.

8. Assay Procedure

Note: This Assay requires utilizing this document in conjunction with the IdentiClone Dx *IGH* Software IFU.

Figure 2. Assay workflow diagram



8.1. Master Mix Preparation

- Include run controls (Positive, Negative, and No Template Controls) with every run.
- One sample replicate is required for testing with each master mix.

8.1.1. Remove the master mix(es), positive control, negative control, NTC and diluted gDNA Sample (in 50 ng/µL) from storage and allow the tube(s) to thaw completely at room temperature; then vortex at maximum speed for approximately 15 seconds

8.1.2. Remove the Taq DNA Polymerase from storage and keep it cold (i.e., over ice or on a cold block).

IMPORTANT! Do NOT Vortex Taq DNA Polymerase enzyme tube.

8.1.3. Using a microcentrifuge, centrifuge tube(s) for 2 to 5 seconds.

Note: **Centrifugation steps are required to collect all liquid at the bottom of a tube or plate well. Approximate speeds and times may be used when successful collection is confirmed visually by the user. Listed speeds and durations are included as a recommendation.**

8.1.4. Use guidelines below to calculate the total number of reactions (N) for each master mix:

- Include at least one additional reaction volume to account for pipetting error.

n	# of samples
+ 1	Positive Control DNA (*IGH* POS (+))
+ 1	Negative Control DNA (NEG (-))
+ 1	No Template Control (NTC)
+ 1	additional reaction volume
N = (n + 4)	**# of samples + 3 controls + 1 additional reaction volume**

8.1.5. Add the calculated volume for each master mix in separate, labeled tubes, using **N × 45 µL**.

8.1.6. Add the calculated volume of Taq DNA Polymerase to each master mix, using **N × 0.25 µL**.

8.1.7. Vortex the master mix + Taq DNA Polymerase solution(s) at maximum speed for 5 to 15 seconds.

- Do NOT vortex Taq DNA Polymerase prior to addition to each master mix. Only vortex after formulation with the master mix.

8.1.8. Using a microcentrifuge, centrifuge tubes between 2 to 5 seconds.

8.1.9. For each reaction, combine 45 µL of the MM + Taq DNA Polymerase solution(s) with 5 µL of template (diluted gDNA Sample in 50 ng/µL, Positive Control, Negative Control, or NTC) into appropriate individual wells in a PCR plate or tube(s).

8.1.9.1. Pipette several times, then close the tube or seal the plate with cap strips or sealing foil; OR

8.1.9.2. Close the tube or seal the plate with cap strips or sealing foil, then vortex at maximum speed for approximately 15 seconds.

8.1.10. If using PCR tube, by using a microcentrifuge, centrifuge tube(s) for 2 to 5 seconds. If using PCR plate, centrifuge the plate at 1000 RCF for approximately 30 seconds.

8.2. PCR Amplification

8.2.1. Amplify the tube(s) or plate according to the PCR parameters provided in Table 6.

8.2.1.1. Ensure that the *Reaction Volume* setting is **50 µL** and the *Cover Temperature* is set to **105°C**.

Table 6. **PCR Parameters**

Step	Temperature	Time	Cycles	Ramp Rate
1	95°C	7 minutes	1	75%
2	95°C	45 seconds	35	
3	60°C	45 seconds		
4	72°C	90 seconds		
5	72°C	10 minutes	1	
6	15°C	∞	Hold	

8.2.1.2. Ensure that the *Reaction Volume* setting is **50 µL** and the *Cover Temperature* is set to **105°C**.

8.2.2. After the PCR program is complete, remove the amplification plate or tubes from the thermal cycler.

8.2.2.1. PCR products can be stored at 2°C to 8°C up to 7 days or stored at -30°C to -15°C up to 3 months prior to detection.

8.2.3. Follow the *Create Platemap* section in the Software IFU to create a platemap before continuing to section 8.3.

8.3. Prepare Detection Plate

Note: **Ensure that the platemap has been created per the *Create PlateMap* section in the Software IFU.**

8.3.1. Allow an appropriate number of Hi-Di Formamide tubes to thaw at room temperature.

- Hi-Di Formamide, 3500 Dx Series unit size is 5 mL.

Note: **To avoid freeze/thaw cycles, the Hi-Di Formamide can be thawed, mixed, and aliquoted into 2 mL tubes at a volume of 1 mL and stored according to manufacturer's instructions.**

8.3.2. Remove a tube of LIZ Size Standard v2.0 Dx from storage.

8.3.3. Vortex both Hi-Di Formamide and LIZ Size Standard at the max speed for 5 to 15 seconds.

8.3.4. Centrifuge the tubes approximately 2 to 5 seconds.

8.3.5. In a new volume-appropriate tube, combine the required amount of Hi-Di Formamide with LIZ Size Standards.

8.3.5.1. The final ratio must be **0.056** (56 µL of LIZ Size Standard + 1000 µL of Hi-Di Formamide).

- Ensure volume of LIZ:Hi-Di solution is sufficient to aliquot 19 µL per reaction.

8.3.5.2. Vortex the solution at the max speed for 5 to 15 seconds, then centrifuge for 2 to 5 seconds.

8.3.6. Centrifuge the amplification plate or tubes from section 8.2.2 at 1000 RCF for 30 seconds.

8.3.7. In a new 96-well PCR plate, combine 19 µL LIZ:Hi-Di solution with 1 µL of PCR product per well.

- Verify only one type of reaction template (*i.e.,* sample, Positive Control, Negative Control, No Template Control) is present per well.

8.3.8. Add 20 µL Hi-Di Formamide to any empty wells within the 24 well injection.

- Each injection on the ABI 3500xL Dx or ABI 3500xL Genetic Analyzer instrument assesses 24 wells at a time.

8.3.9. Mix the detection plate by either:

8.3.9.1. Pipette up and down several times, then seal the plate with cap strips or sealing foil; **OR**

8.3.9.2. Seal the plate with cap strips or sealing foil, then vortex at maximum speed for 15 seconds.

8.3.10. Centrifuge the plate at 1000 RCF for 30 seconds.

8.3.11. Denature the detection plate using the parameters in Table 7 on the thermal cycler.

Note: Refer to section 8.3.1 to confirm ABI reagent volumes are sufficient for the run.

Table 7. Denature Parameters

Step	Temperature	Time	Cycles	Ramp Rate
1	95°C	3 minutes	1	75%
2	4°C	5 minutes	1	

8.3.12. Ensure that the *Reaction Volume* setting is **20 µL** and the *Cover Temperature* is set to **105°C**.

8.3.13. Start the run.

8.3.14. After the denaturing program is complete, remove the detection plate from the thermal cycler, and centrifuge the plate at 1000 RCF for 30 seconds.

8.4. Install Assay Parameters

Note: Section 8.4 is required for the first use of this Assay with any ABI instrument.

Note: Confirm Assay Parameters are set correctly before proceeding to section 8.5.

IMPORTANT! **Instrument and Sizecalling Protocol Parameters will determine how the samples will be processed (i.e., fragment analysis). Ensuring correct parameters are saved with the correct Assay name (*IGH Instrument Parameters*) is essential in proper assay execution.**

8.4.1. Import *IdentiClone Dx IGH Assay parameters* into the ABI instrument.

The Assay parameters may be imported from the provided *IGH Instrument Parameters.xml* file, included in the IdentiClone Dx *IGH* Software Package (REF: 91010111) and requires *ABI 3500 Dx Series Data Collection Software 3 IVD Library* (v3.0 or higher).

8.4.1.1. Double click the **ABI 3500 Dx** icon to open *3500 Dx Series Data Collection Software 3 IVD* (v3.0 or higher).

8.4.1.1.1. From the dashboard menu, click on *Library* (Library).

8.4.1.1.2. Click **Manage** (Manage) then select **Assays** from the dropdown menu.

8.4.1.1.2.1. Select the **Import** icon (Import), then navigate to the file path for the software package and select ***91010111-IP.xml***.

8.4.1.1.3. Click **Manage** (Manage), then select **File Name Convention** from the dropdown menu.

8.4.1.1.3.1. Select the **Import** icon (Import), then navigate to the file path for the software package and select ***91010111-FNC.xml.***

8.4.1.1.4. Click **Manage** (Manage), the select **Results Group** from the dropdown menu.

8.4.1.1.4.1. Select the **Import** icon (Import), then navigate to the file path for the software package and select ***91010111-RG.xml***.

8.4.1.2. Verify that the proper the *Instrument Protocol* (Figure 3) and *Sizecalling Protocol* parameters (Figure 4) are correctly configured and saved in the Assay library on the ABI 3500 Data Collection Software.

Figure 3. Instrument Protocol

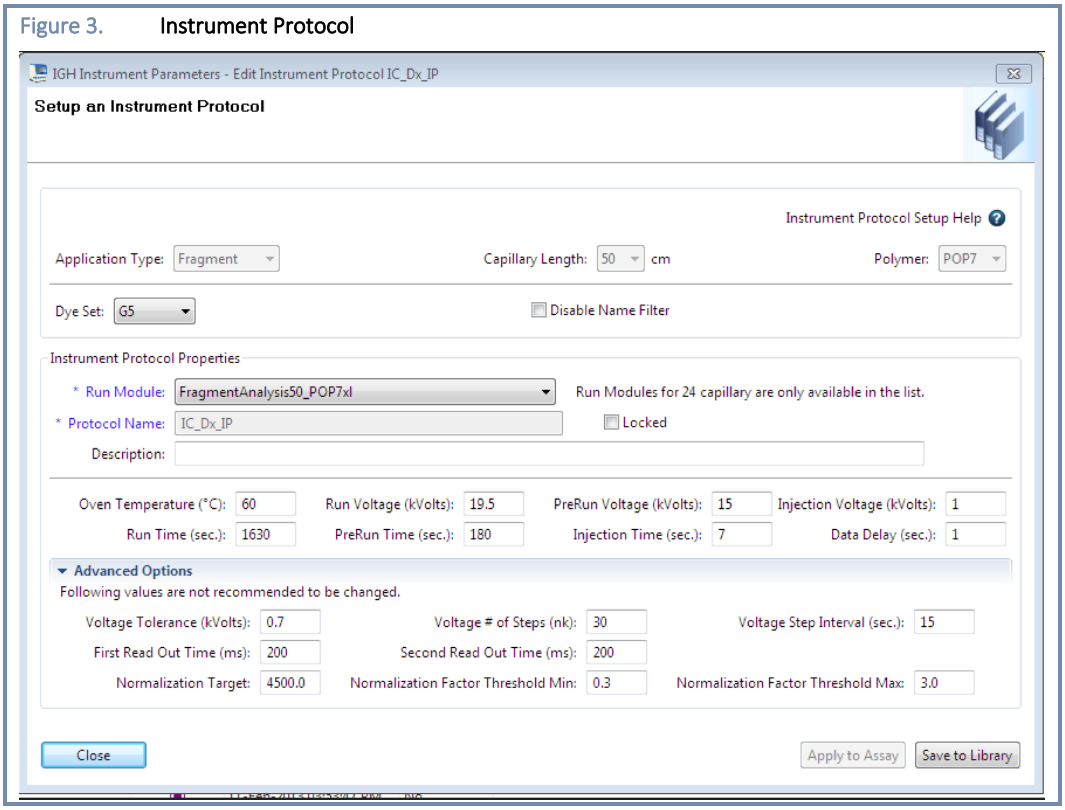
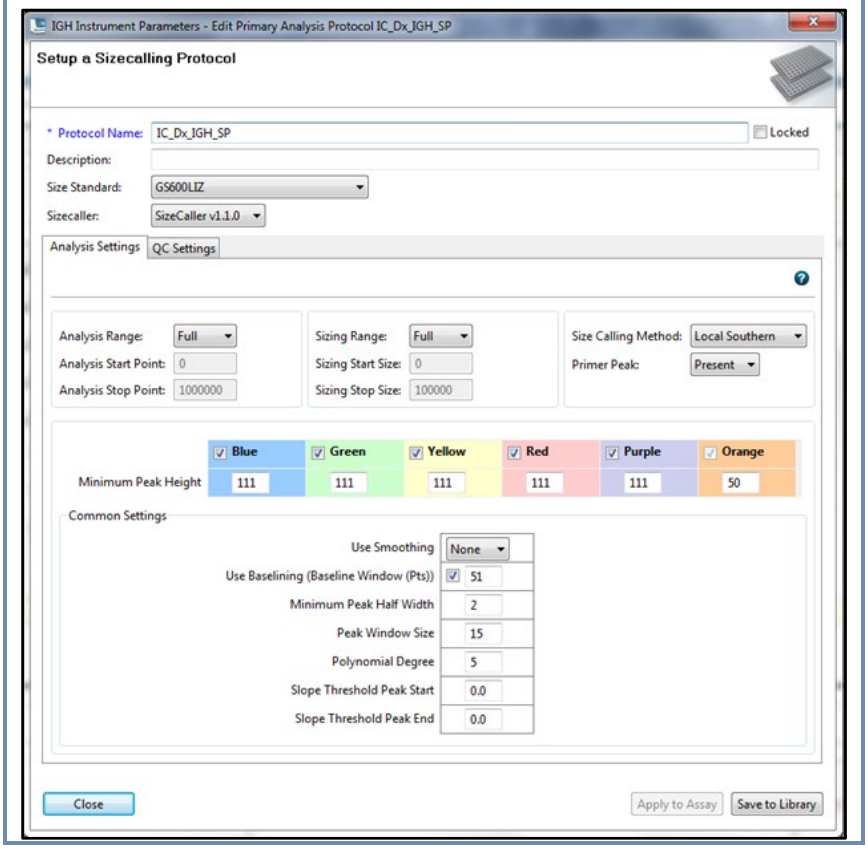
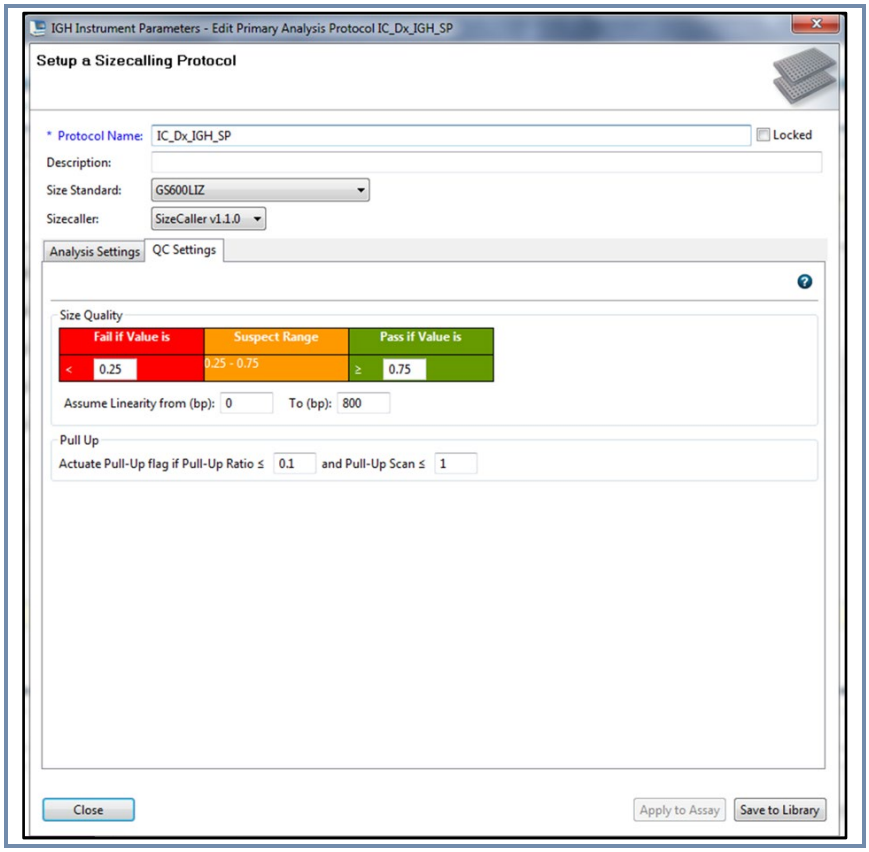


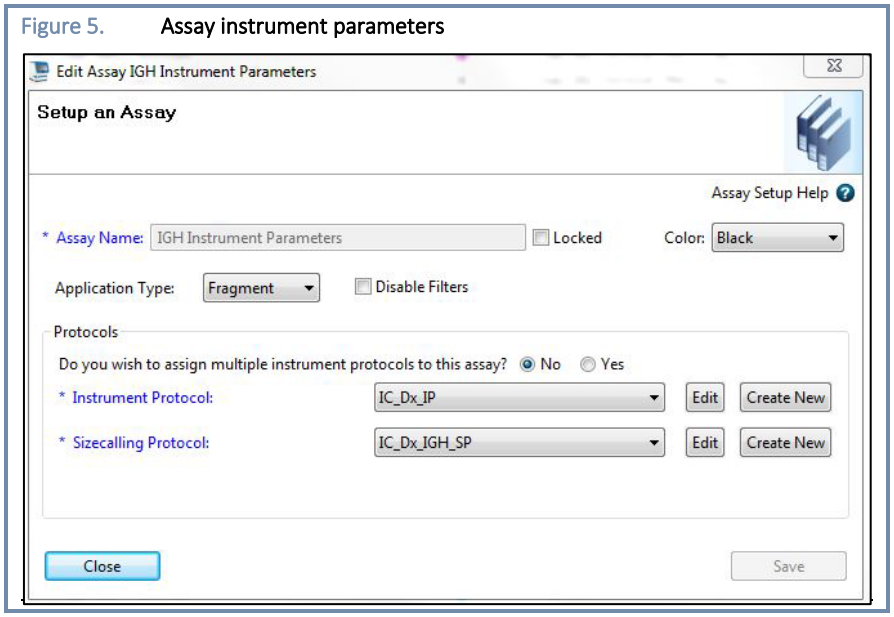
Figure 4. Size Scaling Protocol tabs





8.4.1.3. Save the Assay parameters in the ABI 3500 Dx assay library using the name ***IC_Dx_IP*** for the Instrument Protocol and ***IC_Dx_IGH_SP*** for the Sizecalling Protocol, **exactly** as shown in Figure 5.

Figure 5. Assay instrument parameters



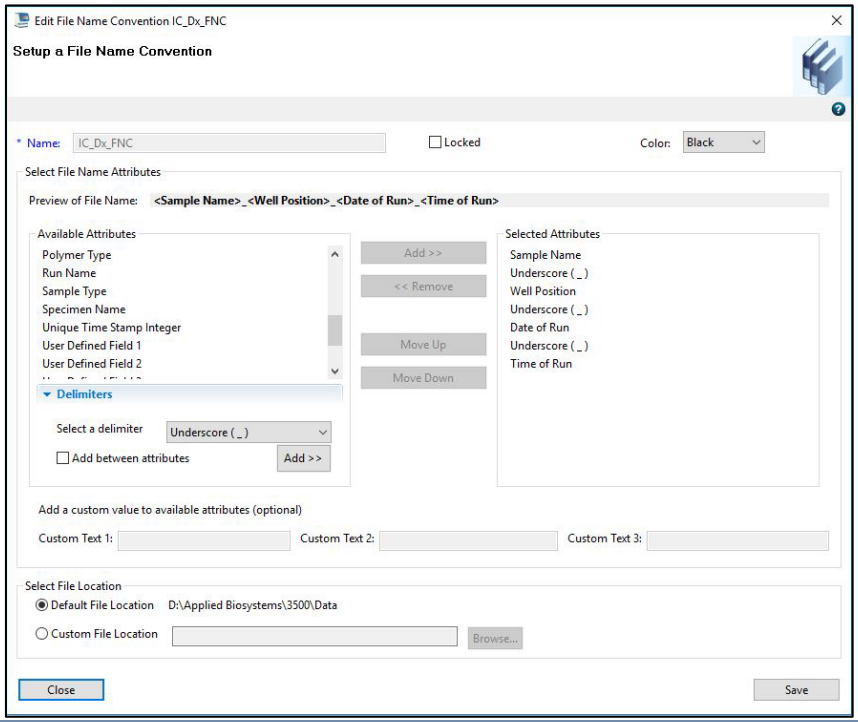
8.4.2. From the *File Name Conventions* section, click on the **pencil** icon  to ensure the *File Name Convention* (FNC) settings match those in Figure 7 and is saved as **IC_Dx_FNC** (**exactly** as shown).

8.4.2.1. The FNC determines which fields of data and by what order the resulting data files (FSA files) will contain.

8.4.2.2. Verify the selected attributes match the order shown in Figure 7.

IMPORTANT: Confirm the *Sample Name* field is first on the list of the *Selected Attributes*.

Figure 6. *File Name Convention* attributes



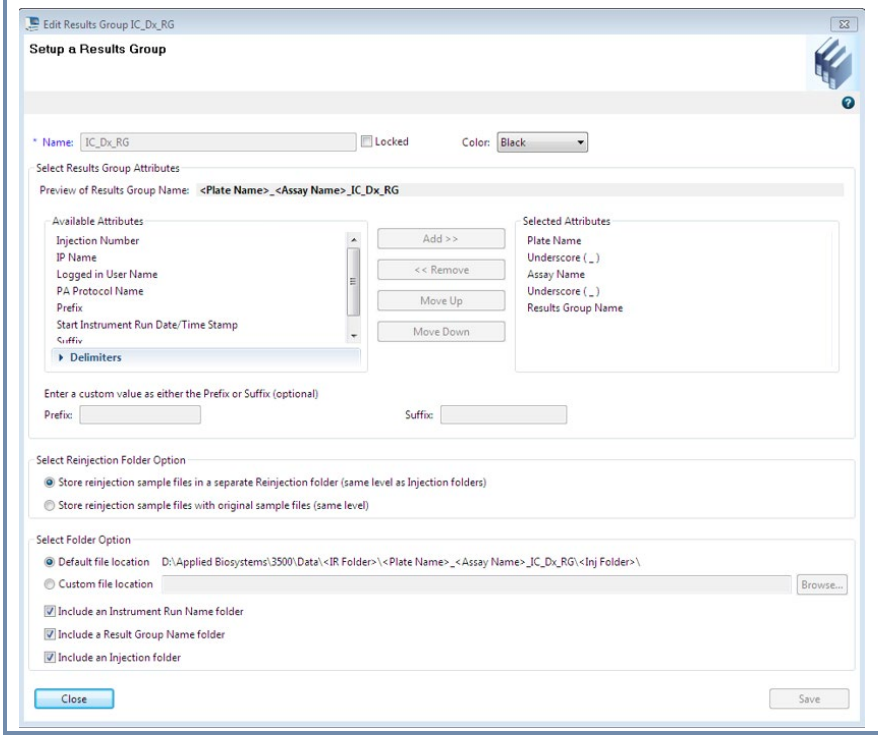
8.4.3. From the *Results Group* section, click on the **pencil** icon  to ensure the *Results Group* (RG) settings match those in Figure 7 and is saved as **IC_Dx_RG** (**exactly** as shown).

8.4.3.1. The Results Group is used to name, sort, and customize the folders in which sample data files are stored.

8.4.3.2. Verify the selected attributes match the order shown in Figure 7.

IMPORTANT: Set up the File Name Convention and Result Group correctly as stated above to avoid data analysis error.

Figure 7. *Results Group* attributes



8.5. Fragment Analysis by Capillary Electrophoresis

8.5.1. Review instrument consumable status.

8.5.1.1. From the ABI instrument dashboard, click **Refresh**, then review the instrument time remaining and number of injections performed for the consumables (Figure 8), and verify the following:

8.5.1.1.1. Sufficient ABI reagent volumes for the capillary electrophoresis (CE) run(s),

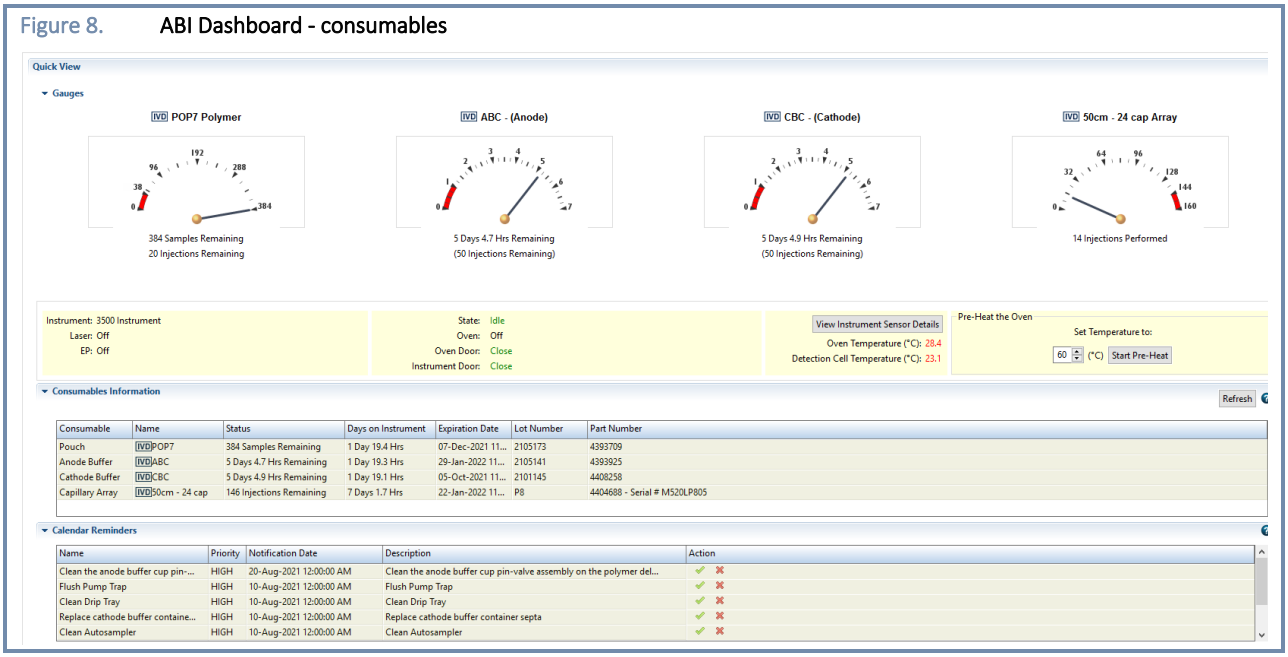
8.5.1.1.2. Buffers, polymer, and capillary have not exceeded the maximum allowable time on the instrument,

8.5.1.1.3. The number of injections remaining for each component is sufficient for the number of injections required for the CE run(s), and

8.5.1.1.4. The remaining POP7 is sufficient for the number of samples in the run(s).

8.5.1.2. If a consumable is expired or needs to be replaced, follow the manufacturer's instructions to perform the necessary maintenance before proceeding.

Figure 8. **ABI Dashboard - consumables**



8.5.2. Import the completed ABI platemap (CSV) created with the IdentiClone Dx *IGH* Software (refer to the *Create Platemap* section in the Software IFU).

IMPORTANT! **Ensure a LIVS file was created when the Platemap was generated. If the LIVS file is missing, IdentiClone Dx *IGH* Software will NOT be able to perform the Result Analysis.**

8.5.2.1. Click **Create New Plate** and enter a name for the plate.

8.5.2.2. Select **96** as the *Number of Wells*.

8.5.2.3. Select **Fragment** as the *Plate Type*.

8.5.2.4. Select **50cm** as the *Capillary Length*.

8.5.2.5. Select **POP7** as *Polymer Type*.

8.5.2.6. Click **Assign Plate Contents**.

8.5.2.7. Click **Import**.

8.5.3. Verify correct platemap attributes were imported.

8.5.3.1. Open the ABI platemap (CSV) created with the IdentiClone Dx *IGH* Software.

8.5.3.1.1. Verify all sample and control wells are named correctly and each well is assigned the correct *Assay*, *File Name Convention*, and *Results Group* (see section 8.4.2 and 8.4.3, respectively).

8.5.3.2. If the plate map does not match the intended setup, generate a new ABI platemap file, starting from section 8.5.2.

IMPORTANT! **Do NOT modify the platemap using the ABI Genetic Analyzer. Use the Software to modify the platemap to ensure the associated LIVS file is also updated.**

If the platemap and LIVS files are misaligned, the Software will NOT be able to perform the Result Analysis.

8.5.3.3. Select any wells that do not contain a reaction (sample or control), *right click*, and select **delete** to prevent the generation of results.

Note: Failure to complete this step may cause SQ errors and/or damage the capillary.

8.5.3.4. Click **Save Plate**, then click **Link Plate for Run**.

8.5.4. Run the plate on the ABI 3500xL Dx or ABI 3500xL Genetic Analyzer instrument.

8.5.4.1. Load the appropriate detection plate (prepared in section 8.3) on the ABI instrument according to the manufacturer instructions.

8.5.4.1.1. Verify that all occupied wells in the detection plate are free of air bubbles and contents are at the bottom of the well.

8.5.4.1.2. Verify that the plate orientation is correct when placed in the instrument.

8.5.4.1.3. Verify that the plate position on the ABI instrument is correctly selected (Plate A vs Plate B).

8.5.4.2. Click **Start Run**.

- The instrument will perform initialization, and if all the internal quality control checks result with a pass status, the run will begin.

8.5.4.2.1. Ensure the run has begun; verify the background of the set of samples in the first injection on the plate display turns green.

8.5.5. Prepare run data for analysis

8.5.5.1. Following the ABI run, verify the run completed without errors, then navigate to the file location containing the run results in FSA format.

- The FSA file location is determined by the *Results Group*, saved in section 8.4, Figure 5.

8.5.5.1.1. Copy the FSA files to the same location containing the LIVS files generated by the Software.

- The LIVS file location is specified during *Plate Setup* (refer to the Software IFU) at the time the platemap was saved.

8.5.5.1.2. Proceed to data analysis using the IdentiClone Dx *IGH* Software, see the *Result Analysis* section in the corresponding Software IFU.

8.5.5.2. Remove the plate from the ABI instrument base and discard.

8.6. Quality Control

Positive, negative and no template controls are furnished with the kit and must be included each time the Assay is performed. Data generated by the Assay will be interpreted by the IdentiClone Dx *IGH* Software, as described in section 9.

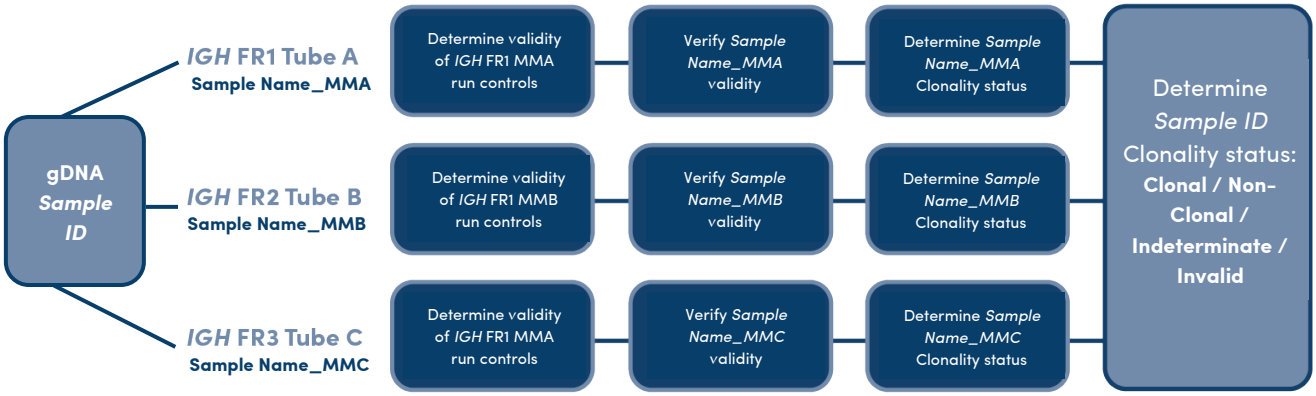
9. Interpretation of Results

9.1. Software Check Points

The IdentiClone Dx *IGH* Software interprets the data generated from the ABI instrument following predetermined logic that requires validity check points to continue with the subsequent analysis step (see Figure 9). Sample clonality status (i.e., *Sample ID* result) requires at least one result from a valid run for each master mix (i.e., *Sample Name* results).

Figure 9: Assay interpretation diagram

- Results from 3 *Sample Names* are combined to determine the final clonality result for the *Sample ID*.
- The final clonality result requires a *Sample Name* result from 1 valid run (for each master mix) generated from each of the 3 master mixes. These results must be from a valid run and can include Invalid and Indeterminate results.
- The *Sample Name* assigned for each master mix are tied to the *Sample ID*.



9.1.1. Run controls are evaluated to determine the run validity.

A valid run status requires the 3 types of run controls (positive, negative and no template) to generate valid results; otherwise, the run status is invalid. All *Sample Name* results included in an invalid run will be automatically considered invalid and analysis will not proceed.

IMPORTANT! Only valid runs, which requires all 3 controls to be valid, will proceed to the next step.

9.1.2. *Sample Name* results from a valid run are evaluated for validity (per individual master mix).

9.1.2.1. An invalid *Sample Name* result will not proceed with further analysis (i.e., master mix clonality status).

9.1.3. Valid *Sample Name* results are evaluated for Clonality (per individual master mix).

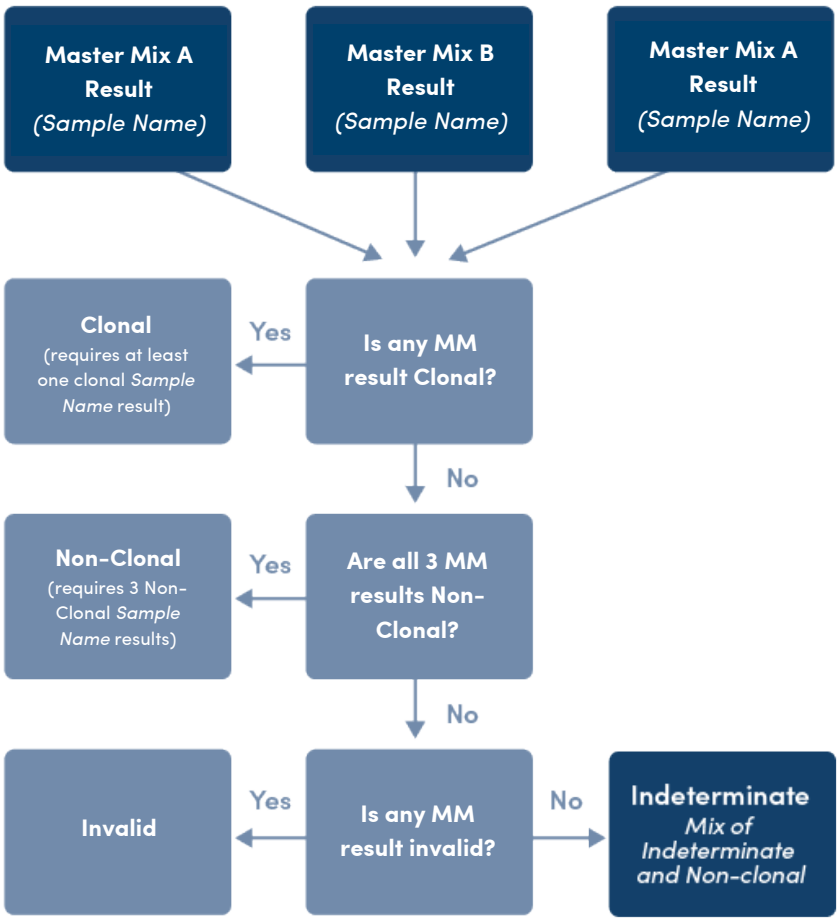
9.1.3.1. Clonal
9.1.3.2. Non-Clonal
9.1.3.3. Indeterminate

9.1.4. Valid *Sample Name* results for all 3 master mixes are assessed to determine the sample clonality status (*Sample ID* result), see Figure 10.

9.1.4.1. If one or more *Sample Name* (or sample-master mix) results are Clonal, the *Sample ID* result is **Clonal**.
9.1.4.2. If all *Sample Name* (or sample-master mix) results are Non-Clonal, the *Sample ID* result is **Non-Clonal**.
9.1.4.3. If *Sample Name* (or sample-master mix) results are mixture of Indeterminate and Non-Clonal results, the *Sample ID* result is **Indeterminate**.
9.1.4.4. If any *Sample Name* (or sample-master mix) result is Invalid and other results are Non-Clonal or Indeterminate, the *Sample ID* result is **Invalid**.

9.1.4.4.1. Invalid *Sample Name* (or sample-master mix) results from a valid run may be retested per section 10 to resolve the *Sample ID* clonality status.

Figure 10. Sample interpretation scheme



10. Retests, if applicable

10.1. Invalid Runs

10.1.1. A run in which any of the controls do not meet validity criteria is an **Invalid Run**. Repeat the run including all samples, Positive control, Negative Control and NTC. Each master mix is run independently.

10.1.2. Repeat the run according to the associated Software IFU, based on the Error Code(s) listed in the IdentiClone Dx *IGH* Software Run Report.

10.2. Invalid Samples within a Valid Run

Invalid *Sample Name* results generated from a valid run require retesting if the *Sample Name* results of the other master mixes include Non-Clonal and/or Indeterminate; otherwise, retesting is not required. See Figure 11 for the retesting hierarchy.

10.2.1. If results from any of the master mixes are **Clonal** (within a valid run), no retest is required.

10.2.2. If results from all master mixes are **Non-Clonal** (within valid runs), no retest is required.

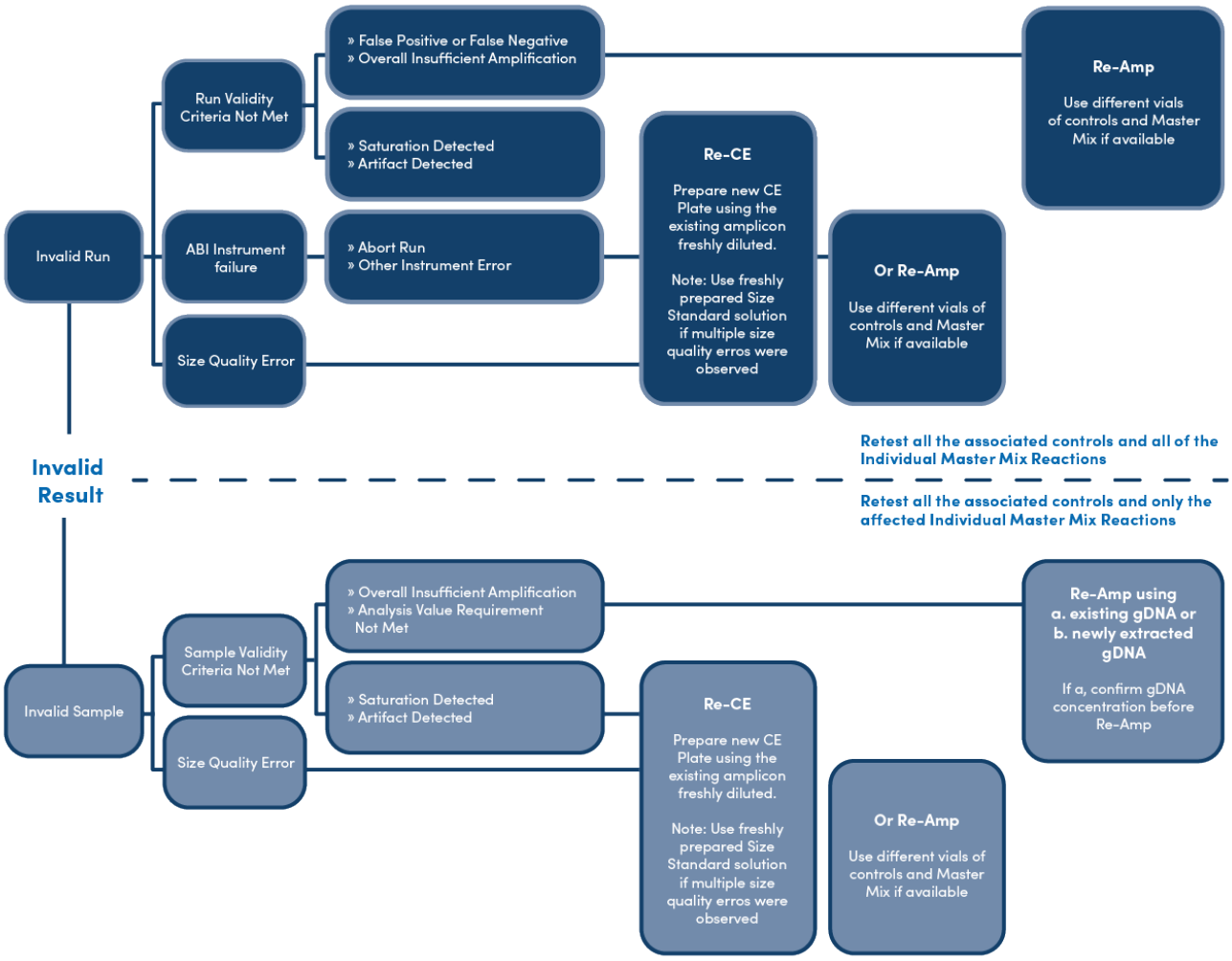
10.2.3. If results from all master mixes are **Non-Clonal, Indeterminate**, and **Invalid**, retest the *Sample Name(s)* with the Invalid result and the associated run controls according to the error code (see Figure 11).

10.3. Fail Details and Retesting

Up to four retesting events are allowed when the failures are due to different cause (i.e., 1st round ⇨ Invalid *Sample Name* result due to Invalid run; 2nd round ⇨ Invalid *Sample Name* result due to artifact; 3rd round ⇨ Invalid *Sample Name* result due to saturation; 4th round ⇨ Invalid *Sample Name* result due to SQ error).

Figure 11. Retest Instruction

Note: Sample in this figure refers to *Sample Name* (individual master mix product)



11. Limitations of Procedure

- Limit of Detection: 2.5% B-cell clonality
- This Assay does not identify 100% of Clonal cell populations.
- Always interpret the results of molecular clonality tests in the context of clinical, histological, and immunophenotypic data.
- PCR-based assays are subject to interference by degradation of DNA or to inhibition of PCR due to EDTA, heparin, or other agents.

12. Performance Characteristics

12.1. Specimen and Sample Validations - Specimen Stability

12.1.1. This study provides a sample type validity evaluation to support the proposed Instructions for Use claims; the investigational device is validated for peripheral blood anti-coagulated with EDTA. The purpose of this study is to determine the stability of peripheral blood anti-coagulated with EDTA specimen type for the IdentiClone Dx *IGH* Assay by providing objective evidence from testing specimens before and after various storage conditions with the investigational device as compared to specimen-specific baseline test results. This study was conducted on 2 replicates of 15 specimens (10 B-cell Clonal/Positive, 5 Non-Clonal/Negative) tested at 5 different timepoints with 1 reagent lot, on multiple ABI 3500xL Dx instruments, by multiple operators over multiple days. Timepoints tested include:

- Timepoint-0: Baseline
- Timepoint-1: Refrigerated (2-8°C) for 5 Days
- Timepoint-2: Refrigerated (2-8°C) for 7 Days
- Timepoint-3: Room Temp (15-30°C) for 5 Days
- Timepoint-4: Room Temp (15-30°C) for 7 Days

12.1.2. Table 8 provides the test result for each replicate tested of the 15 specimens. All sample test results (2 replicates for each of the 15 specimens) were valid (100.0% sample validity rate, 30/30). Both replicates of every Non-Clonal and Clonal specimen generated the expected results, indicating 100.0% concordance between Timepoint-0 vs. Timepoint-1, Timepoint-0 vs. Timepoint-2, Timepoint-0 vs. Timepoint-3, and Timepoint-0 vs. Timepoint-4.

Table 8. Clonality Status Result Summary

Subject ID	Repli-cate	Clonality Status				
		Timepoint 0	Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
CS189	1	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
	2	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
030723-1	1	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
	2	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
030723-2	1	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
	2	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
041223-4	1	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
	2	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
CS231	1	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
	2	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal	Non-Clonal
CS201	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS08	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS09	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS10	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS11	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS12	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS13	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS14	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal

Table 8. **Clonality Status Result Summary**

Subject ID	Repli-cate	Clonality Status				
		Timepoint 0	Timepoint 1	Timepoint 2	Timepoint 3	Timepoint 4
DLS15	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal
DLS16	1	Clonal	Clonal	Clonal	Clonal	Clonal
	2	Clonal	Clonal	Clonal	Clonal	Clonal

12.1.2.1. The results of this study have established the stability of peripheral blood anti-coagulated with EDTA specimen type for use with the IdentiClone Dx *IGH* Assay to be ≥5 days when stored at 2°C - 8°C or room temperature (15°C to 30°C).

12.2. Clinical Cut-Off

12.2.1. This study performs a comparative assessment of historical data generated from the IdentiClone Dx *IGH* Assay with clinical samples, previously identified as positive for clonal *IGH* gene rearrangements, to establish the clinical cutoff value of the relative peak ratio (RPR). The two reference methods selected include 1) a commercially available IVD assay (at the time of the study) that applies next generation sequencing (NGS) to detect clonal *IGH* gene rearrangements (Reference Assay), with an intended use similar to the investigational device, the IdentiClone Dx *IGH* Assay, and 2) the Clinical Diagnosis based on the ICD-10 codes for B-cell lymphoproliferative disease (assigned Clonal) or Healthy (assigned Non-Clonal).

12.2.2. Overall, results from 170 specimens were evaluated. The first data set included 114 samples that generated a valid result with both the Reference Assay (IVD) and the IdentiClone Dx *IGH* Assay (IUO); these were evaluated for percent (%) agreement of the results generated by the two assays. Evaluation was performed using 3 different RPR values as the cutoff: 2.0, 3.0 and 4.0. The second dataset included 145 samples that generated a valid result with the IdentiClone Dx *IGH* Assay in conjunction with a known clinical diagnosis based on the ICD-10 code or healthy status. This data set was evaluated for % agreement between the 2 classifications (Clonal or Non-Clonal) using a cutoff value of 3.0. Different subsets of test results from the 170 specimens were used for evaluation because some specimens generated invalid results with the IdentiClone Dx *IGH* Assay (IUO) or the Reference Assay (IVD), or because clinical diagnosis information (ICD-10 code or healthy status) was not available.

12.2.2.1. The first dataset of 114 samples compared clonality results generated by the IdentiClone Dx *IGH* Assay to the Reference Assay using the RPR value set at ≥3.0 as the cutoff. OPA, PPA and NPA against the Reference Assay were 92.1%, 93.8% and 87.9%, respectively. The lower limit of 95% CI calculated using the two-sided score method for PPA and NPA were 86.4% and 72.7%, respectively. (Table 9) To ensure the robustness of a clinical cutoff RPR value of 3.0, the same data was reanalyzed using RPR values both below and above an RPR = 3.0 (i.e., RPR = 2.0 and RPR = 4.0). The clinical cutoff RPR value set at ≥3.0 was shown to be optimal since it generated best % agreement value between the Investigational Device and the Reference Assay, with wider gap away from the RPR value ranked immediately lower.

Table 9. **Evaluation against the Reference Assay (IVD)**

IdentiClone Dx *IGH* Assay (IUO)	Reference Assay (IVD)		Total
	Clonal	Non-Clonal	
Clonal	76	4	**80**
Non-Clonal	5	29	**34**
Total	81	33	**114**
OPA	92.1%		
PPA	93.8% (95% CI: 86.4% - 97.3%)		
NPA	87.9% (95% CI: 72.7% - 95.2%)		

12.2.2.2. The second dataset comprised of 145 samples and compared a known clinical diagnosis (ICD-10 code or healthy status) to the clonality results generated with the IdentiClone Dx *IGH* Assay using an RPR value of ≥3.0 as the cutoff. The resulting OPA, PPA and NPA against the clinical diagnosis were 91.7%, 92.8% and 90.3%, respectively. LL 95% CI calculated using the two-sided score method for PPA and NPA were 85.1% and 80.5%, respectively. (Table 10)

Table 10. **Evaluation with Clinical Diagnosis as the Reference Method**

IdentiClone Dx *IGH* Assay (IUO)	Clinical Diagnosis (Reference)		Total
	B-Cell Lymphoproliferative Disease	Healthy	
Clonal	77	6	**83**
Non-Clonal	6	56	**62**
Total	**83**	**62**	**145**
OPA	91.7%		
PPA	92.8% (95% CI: 85.1% - 96.6%)		
NPA	90.3% (95% CI: 80.5% - 95.5%)		

12.2.3. The clinical sample test results generated by the IdentiClone Dx *IGH* Assay demonstrate high concordance to the reference methods, using clinical cutoff RPR value set at ≥3.0, which is also the cutoff RPR value for determining clonality. The LL of 95% CI for PPA and NPA were both above 70% when compared to the NGS-based Reference Assay, a commercially available IVD assay, or when compared to the clinical diagnosis.

12.3. Analytical Sensitivity: Limit of Blank (LoB)

12.3.1. This study establishes the Limit of Blank (LoB) for the IdentiClone Dx *IGH* Assay by testing 20 specimens from Non-Clonal donors (3 replicates each) with 2 reagent lots on multiple ABI 3500xL Dx Genetic Analyzers, by multiple operators over multiple days (60 data points per master mix, per reagent lot).

12.3.2. All (100%) of the tested LoB Test Panel samples were Non-Clonal (Negative) with RPR < 3.0. The highest RPR observed (2.9) was generated from testing a negative clinical specimen with MMA, Reagent Lot 2. For each reagent lot, 60 test results were ranked by the RPR. The 95-percentile responds to 57.5th rank; thus, the average of the 57th and 58th-ranked RPR for each master mix were calculated for each reagent lot. (Table 11) In conclusion, using the conservative approach, the overall assay LoB is determined to be RPR of 2.0.

Table 11. **LoB Result Summary**

Reagent Lot	MM	Rank	RPR	Average RPR of Ranks 57 + 58
1	A	57	1.7	1.8
		58	1.8	
	B	57	1.9	2.0
		58	2.0	
	C	57	1.7	1.7
		58	1.7	
2	A	57	1.8	1.9
		58	1.9	
	B	57	1.8	1.9
		58	1.9	
	C	57	1.8	1.9
		58	1.9	

12.4. Analytical Sensitivity: Limit of Detection (LoD)

12.4.1. This study establishes a limit of detection (LoD) for the IdentiClone Dx *IGH* Assay by providing objective evidence from results generated with clinical gDNA extracted from peripheral blood representing the *IGH* clans (I, II and III) diluted with gDNA from pooled peripheral blood (PPB) obtained from healthy donors.

12.4.2. A total of 20 specimens (10 healthy donors and 10 donors suspected of B-cell lymphoproliferations with Non-Clonal status) comprised the PBB used to prepare the 7 dilutions of gDNA representing each *IGH* clan (I/II/III) used for testing with the Investigational Device: 0.0%, 0.3%, 1.0%, 3.0% and 10.0%. The resulting RPR values from samples generating a Clonal result were verified against the Clonality Standard Table (Table 12), which provides the correlation between the RPR value generated for each master mix with each *IGH* clan to the % dilution of the clonal *IGH* rearrangement. A minimum of 20 valid results were generated with the IdentiClone Dx *IGH* Assay for each *IGH* clan at each dilution, with each reagent lot. This study was conducted with 2 reagent lots, on multiple ABI3500xL Dx instruments, by multiple operators over multiple days.

Table 12. **Clonality Standard Table**

% Dilution or % Clonality	Master Mix RPR Values							
	IGH clan I[A]			*IGH* clan II[B]		*IGH* clan III[C]		
	MMA	MMB	MMC	MMA	MMB	MMA	MMB	MMC
25%	65.2	121.9	131.5	91.2	119.5	149.7	169.1	162.8
10%	52.6	96.0	92.5	26.5	40.5	53.0	57.3	79.9
5%	30.4	48.0	50.4	12.1	18.1	28.7	31.8	34.2
2.5%	14.9	25.7	29.9	6.3	8.6	11.8	11.7	15.9
1.0%	5.1	10.8	13.3	2.3	3.0	3.9	4.8	7.1
0.5%	2.9	4.7	5.9	1.3	1.6	1.8	2.0	3.3
0.25%	1.4	2.3	3.1	1.3	1.3	1.4	1.3	1.8
0.1%	1.4	1.4	1.4	1.3	1.4	1.3	1.5	1.5
0.05%	1.3	1.4	1.3	1.5	1.3	1.2	1.2	1.4

[A]IVS-0019 cell line used as clonal standard for *IGH* clan I clonality estimation
[B]IVS-0002 cell line used as clonal standard for *IGH* clan II clonality estimation
[C]IVS-0003 cell line used as clonal standard for *IGH* clan III clonality estimation

12.4.2.1. The 1.0% dilution of *IGH* clan I amplified with Master Mix C (Reagent Lot 1 - 95% positive, average RPR = 4.1) and the 3.0% *IGH* clan 1 dilution with MMA, MMB and MMC (Reagent Lot 2 MMA - 100% positive, average RPR = 9.9; Reagent Lot 2 MMB - 100% positive, average RPR = 12.8; Reagent Lot 2 MMC - 100% positive, average RPR = 13.2) are the lowest % dilution with % positive of ≥ 95% per reagent lot of master mix. (Table 13 and Table 14). Since the LoD is to be established based on the worst performance, the higher % dilution result from the two reagent lots (RPR values generated by the 3.0% dilution of *IGH* clan I with reagent lot 2) were selected for evaluation. The RPR values generated by the 3.0% *IGH* clan I dilution with each master mix were evaluated against the Clonal Standard Table to convert the % dilution to the % clonality.

12.4.2.1.1. According to the Clonal Standard Table, MMA, MMB and MMC RPR values fall within the range corresponding to the 1.0% - 2.5% clonality range for MMA (RPR range = 5.1 - 14.9), 1%-2.5% clonality range for MMB (RPR range = 10.8 - 26.7) and 0.5%-1% clonality range for MMC (RPR value range = 5.9 - 13.3). Using a conservative approach, the LoD for *IGH* clan I was determined to be 2.5% clonality.

12.4.2.2. The 3.0% dilution of *IGH* clan 2 amplified with MMB is the lowest % dilution where % positive of ≥ 95% was observed (reagent lot - 100% positive, average RPR = 8.5; reagent lot 2 - 100% positive, average RPR = 8.3). RPR values of 8.5 and 8.3 for MMB correlate to 1.0% - 2.5% clonality, as per the Clonal Standard Table. Thus, the LoD for *IGH* clan 2 is determined to be 2.5% clonality.

12.4.2.3. The lowest % dilution where the % positive of ≥ 95% that was observed for *IGH* clan III is the 1.0% dilution with MMC (100% positive, average RPR=6.3) with both reagent lots. Using the clonality standard table, an RPR of 6.3 falls within a range of RPR values that correspond to a range of 0.5% - 1.0% clonality. Hence, the LoD for *IGH* clan III is determined as 1.0% clonality.

12.4.3. Given that the LoD for *IGH* clan I, II and III were determined to be 2.5%, 2.5% and 1% clonality, and applying a conservative approach, the overall assay LoD is determined to be 2.5%.

Table 13. **LoD Result Summary for Reagent Lot 1**

Clan	% Dilution	MMA			MMB			MMC		
		valid n	% pos	Avg RPR	valid n	% pos	Avg RPR	valid n	% pos	Avg RPR
N/A	0.0	19	0	1.4	20	0	1.4	20	0	1.4
I	0.1	20	0	1.4	20	0	1.4	20	0	1.4
	0.3	20	0	1.3	20	0	1.4	20	0	1.3
	1.0	18	50.0	3.0	19	89.5	3.8	20	95.0	4.1
	3.0	20	100.0	10.2	20	100.0	13.3	20	100.0	12.9
	10.0	19	100.0	40.3	20	100.0	52.7	20	100.0	45.9
	30.0	20	100.0	91.4	20	100.0	144.5	20	100.0	111.2

Table 13. LoD Result Summary for Reagent Lot 1

Clan	% Dilution	MMA			MMB			MMC		
		valid n	% pos	Avg RPR	valid n	% pos	Avg RPR	valid n	% pos	Avg RPR
II	0.1	20	0	1.4	20	0	1.5	20	0	1.4
	0.3	20	0	1.3	20	0	1.6	20	0	1.4
	1.0	19	0	1.3	20	50.0	2.9	20	0	1.3
	3.0	19	0	1.9	20	100.0	8.5	20	0	1.4
	10.0	20	100.0	6.9	20	100.0	30.7	19	0	1.3
	30.0	20	100.0	28.8	20	100.0	99	20	0	1.3
III	0.1	20	0	1.4	20	0	1.4	20	0	1.3
	0.3	20	0	2.0	20	0	1.5	20	0	1.9
	1.0	20	100.0	5.8	20	95.0	4.4	19	100.0	6.3
	3.0	20	100.0	17.5	19	100.0	14	20	100.0	19.2
	10.0	20	100.0	59.3	20	100.0	52.8	20	100.0	67.6
	30.0	20	100.0	113.9	20	100.0	132.5	20	100.0	147.4

Table 14. LoD Result Summary for Reagent Lot 2

IGH Clan	% Dilution	MMA				MMB				MMC			
		valid n	n clonal	% pos	Avg RPR	valid n	n clonal	% pos	Avg RPR	valid n	n clonal	% pos	Avg RPR
N/A	0.0	20	0	0.0	1.4	20	0	0.0	1.4	20	0	0.0	1.4
I	0.1	20	0	0.0	1.3	20	0	0.0	1.4	20	0	0.0	1.3
	0.3	20	0	0.0	1.3	20	0	0.0	1.4	20	0	0.0	1.5
	1.0	20	10	50.0	3.0	19	16	84.2	3.8	19	17	89.5	3.9
	3.0	18	18	100.0	9.9	20	20	100.0	12.8	20	20	100.0	13.2
	10.0	20	20	100.0	37.4	20	20	100.0	53.7	19	19	100.0	46.3
	30.0	20	20	100.0	90.4	20	20	100.0	118.7	20	20	100.0	114.9
II	0.1	20	0	0.0	1.3	20	0	0.0	1.4	20	0	0.0	1.4
	0.3	20	0	0.0	1.4	20	0	0.0	1.6	20	0	0.0	1.4
	1.0	20	0	0.0	1.3	20	10	50.0	3.1	20	0	0.0	1.4
	3.0	20	0	0.0	1.7	20	20	100.0	8.3	20	0	0.0	1.4
	10.0	20	20	100.0	6.5	20	20	100.0	28.9	19	0	0.0	1.3
	30.0	20	20	100.0	28.3	20	20	100.0	82.8	20	0	0.0	1.4
III	0.1	20	0	0.0	1.4	20	0	0.0	1.3	20	0	0.0	1.4
	0.3	20	0	0.0	2.1	20	0	0.0	1.7	20	0	0.0	2.1
	1.0	19	19	100.0	6.1	19	18	94.7	5.2	20	20	100.0	6.3
	3.0	20	20	100.0	18.7	20	20	100.0	14.6	20	20	100.0	18.9
	10.0	19	19	100.0	65.9	20	20	100.0	54.3	20	20	100.0	63.7
	30.0	20	20	100.0	133.9	20	20	100.0	125.7	20	20	100.0	131.5

12.5. Analytical Specificity: Interfering Substances

12.5.1. This study design was based on based on CLSI EP07-A3 and EP37-Ed1 standards and includes evaluating the effect of 6 different, potentially interfering substances when present in specimen tested with the IdentiClone Dx *IGH* Assay. The substances tested were Bilirubin, Hemoglobin, Cholesterol, EDTA, Triglyceride, and 70% Ethanol, which was spiked into gDNA samples after extraction since the potential introduction of this substance into the assay workflow would occur after the gDNA extraction. Otherwise, the other substances were spiked into fresh PB specimens before gDNA extraction.

12.5.2. A total of 24 fresh (never been frozen) peripheral blood anti-coagulated in EDTA specimens from normal donors (negative/Non-Clonal) or diagnosed B-cell lymphoproliferative donors (positive/Clonal) were used in this study and were stored at 2°C to 8°C up to 7 days before gDNA extraction. Each specimen was tested in duplicate, unspiked or spiked, comprising of 10 Clonal specimens per substance and 5 Non-Clonal specimens per substance. This study was conducted with 2 reagent lots, on multiple ABI3500xL Dx instruments, by multiple operators over multiple days.

12.5.3. Results of this study are provided in Table 15 and demonstrate no interference by the 6 substances at the tested concentrations. All specimens generated the expected clonality status (Clonal or Non-Clonal) when tested unspiked vs. spiked. Specifically, each interfering substance tested 5 Non-Clonal and 10 Clonal specimens, with exception for the substance cholesterol. Cholesterol was tested with 5 Non-Clonal and 11 Clonal specimens and each un-spiked and spiked samples were tested in duplicate. Overall, 30 clonality results were determined for each interfering substance before and after spiking, except for cholesterol (32 final calls). All clonality status results generated by spiked samples were concordant with the clonality status results generated by the corresponding un-spiked samples, demonstrating that substances tested did not impose interference to the assay at the tested concentration. The lower limit of the 95% confidence interval for the % agreement for each interfering substance was at or greater than 88.4%. (Table 15)

Table 15. Final Call Percent Agreement Un-Spiked vs. Interfering Substance

Interfering Substance (Final Test Concentration)	Sample Type	N	Total Test Results		Total Concordant Result[2]	% Agreement (95% CI)[3]
			Unspiked	Spiked		
Cholesterol (4.0 mg/mL)	Non-Clonal	5	32	32	32	100.0%
	Clonal	11				(89.1-100.0)
Triglyceride (15.0 mg/mL)	Non-Clonal	5	30	30	30	100.0%
	Clonal	10				(88.4-100.0)
Hemoglobin 100 mg/mL)	Non-Clonal	5	30	30	30	100.0%
	Clonal	10				(88.4-100.0)
Bilirubin (0.4 mg/mL)	Non-Clonal	5	30	30	30	100.0%
	Clonal	10				(88.4-100.0)
EDTA (5.4 mg/mL)	Non-Clonal	5	30	30	30	100.0%
	Clonal	10				(88.4-100.0)
70% Ethanol (10% v/v)	Non-Clonal	5	30	30	30	100.0%
	Clonal	10				(88.4-100.0)

[1]Two replicates per each specimen tested before and after spiking with interfering substances.
[2]Spiked test results matching the corresponding Un-spiked test result from same specimens.
[3]Clopper-Pearson Exact method.

12.6. Analytical Specificity: Carryover / Cross Contamination

12.6.1. This study evaluated the rate of carryover and cross-contamination during the IdentiClone Dx *IGH* Assay workflow at PCR setup and capillary electrophoresis fragment analysis. Testing was conducted on 106 contrived negative samples for carryover and 126 contrived negative samples for cross-contamination. Contrived negative (Non-Clonal) samples were prepared with 100% IVS-0000 Polyclonal Control DNA; contrived positive (Clonal) samples were prepared with 100% IVS-0019 Clonal Control DNA.

12.6.2. The plate layout comprised of alternating positive and negative samples in a checkerboard pattern, designed to maximize the occurrence of carryover and cross-contamination. These samples were amplified with master mix A (MMA), chosen as the representative master mix since contamination is amplicon signal dependent, not master mix dependent. Testing was conducted on multiple plates with alternating sample layouts on 2 different ABI 3500xL Dx Genetic Analyzers, by 1 operator, with 1 reagent lot, over multiple days.

12.6.3. Prior to performing capillary electrophoresis fragmentation on test samples, a base line was established by running a plate consisting of a Liz/HIDI solution through the instrument to confirm the absence of any contaminating signal present from previous studies. Carryover was assessed using data generated by negative samples from plates consisting of the same sample layout (10 total injections, 106 combined negative samples). All 105 negative sample results that qualified for carryover evaluation generated Non-Clonal results, and the percent of false calls due to carryover is 0.0% (0/105), with a 95% Lower CI of 96.5%. Cross-contamination was evaluated using data generated from negative samples from plates containing an alternate sample layout (as compared to those assessed for carryover); 12 total injections, 126 combined negative samples; the percent of false calls due to cross-contamination is 0.0% (0/125), with a 95% Lower CI of 97.1%. Of the 129 positive sample results, all were Clonal except for 2 indeterminate results, which both generated high-RFU peaks, as compared to other positive samples, and were determined to be valid and potentially contaminating signals. Positive sample results across two instruments generated an average RPR value of 100.4, and the average RPR value of negative sample results across two instruments that qualified for carryover evaluation and cross-contamination were 1.2 and 1.2, respectively. (Table 16)

Table 16. Average RPR values by ABI Instrument

Type	ABI Instrument	N	Average RPR	%CV
Clonal	1	65	97.8	30.2
	2	62	103.2	36.3
	Combined	127	100.4	33.5
Carryover (Non-Clonal)	1	63	1.2	4.3
	2	62	1.2	5.0
	Combined	125	1.2	4.6
Cross-Contamination (Non-Clonal)	1	53	1.2	4.3
	2	52	1.2	4.8
	Combined	105	1.2	4.6

12.7. Analytical Specificity: Within-Laboratory Precision Study

12.7.1. The precision of the IdentiClone Dx *IGH* Assay was determined by testing the same sample panel by 3 operators, on 3 instruments using 3 reagent lots, over 20 days. The sample panel consisted of 1 negative sample gDNA, prepared from a pool of negative clinical specimen, and 6 positive samples, prepared from positive clinical specimen gDNA mixed with pooled negative clinical specimen gDNA at 1.5X LoD and 3X LoD. Every sample in the panel was tested in triplicate and testing was conducted at least 20 days apart.

12.7.2. All panel test results matched the expected clonality result (100.0%). The negative sample generated Non-Clonal results in all (100.0%) replicates. The low positive samples (1.5X LoD) generated Clonal results in all (100.0%) replicates, and the high positive samples also generated Clonal results in all (100.0%) replicates. The %CV of the RPR value produced by each master mix are provided in Table 17, and the %CV of RPR value generated by the low positive samples with the Clonal master mixes are shown below.

12.7.2.1. RPR value for MMA, the %CV observed was 12.2%

12.7.2.2. RPR value for MMB, the %CV observed was 10.5%

12.7.2.3. RPR value for MMC, the %CV observed was 8.5%

12.7.3. Total variation, between operator, instruments, reagent lots, and within-run variation are shown in Table 17.

12.7.3.1. The Lot factor results produced the greatest within-run variability for all samples (average variability = 7.9%; minimum = 0% and maximum = 42.3%).

12.7.3.2. The Operator factor results generated an average of 3.1% (minimum = 0% and maximum = 21.7%).

12.7.3.3. The % variance attributed to ABI Instrument had an average variance of 2.2% (minimum = 0% and maximum = 8.5%).

12.7.3.4. The Intrinsic factor results attributed to an average variance of 86.9% (minimum = 55.4% and maximum = 99.9%).

12.7.4. Overall, all sample test results matched the expected clonality result (100.0%). RPR value %CVs were ≤ 25 for Low Positive samples with MMA, MMB and MMC, respectively, across 3 operators, 3 instruments and 3 reagent lots.

Table 17. Assay Precision (Within-Lab) Variability

PM	MM	Valid N	Avg RPR	Variation Type				Total Variation	
				Between Operators	Between Instruments	Between Reagent Lots	Within-Run	SD	%CV
PM1	A	52	1.59	0.08 (7.7 %)	0.07 (5.9%)	0.05 (3.3%)	0.26 (83.2%)	0.28	17.7
	B	53	1.67	0.00 (0.0 %)	0.05 (3.3%)	0.06 (3.7%)	0.28 (93.0%)	0.29	17.2
	C	54	1.52	0.06 (7.1 %)	0.04 (3.4%)	0.06 (5.2%)	0.22 (84.3%)	0.24	16.0
PM2	A	54	16.49	0.72 (12.8 %)	0.00 (0.0%)	1.02 (25.7%)	1.58 (61.5%)	2.02	12.2
	B	54	22.09	0.00 (0.0 %)	0.05 (0.1%)	0.00 (0.0%)	2.16 (99.9%)	2.16	9.8
	C	54	1.40	0.00 (0.0 %)	0.05 (8.5%)	0.00 (0.0%)	0.17 (91.5%)	0.17	12.4
PM3	A	54	1.32	0.02 (1.3 %)	0.01 (0.5%)	0.00 (0.0%)	0.18 (98.2%)	0.18	13.4
	B	53	23.57	0.20 (0.7 %)	0.60 (5.9%)	1.53 (38.0%)	1.84 (55.4%)	2.47	10.5
	C	53	1.38	0.04 (5.6 %)	0.00 (0.0%)	0.00 (0.0%)	0.16 (94.4%)	0.17	12.1
PM4	A	53	25.94	0.56 (1.4 %)	0.00 (0.0%)	0.00 (0.0%)	4.72 (98.6%)	4.76	18.3
	B	54	42.10	0.14 (0.0 %)	0.00 (0.0%)	2.41 (13.8%)	6.04 (86.2%)	6.50	15.4
	C	52	37.59	0.00 (0.0 %)	0.41 (1.6%)	0.00 (0.0%)	3.17 (98.4%)	3.20	8.5
PM5	A	54	33.44	0.00 (0.0 %)	0.00 (0.0%)	2.05 (12.4%)	5.46 (87.6%)	5.83	17.4
	B	54	49.18	0.00 (0.0 %)	0.78 (2.3%)	0.00 (0.0%)	5.03 (97.7%)	5.09	10.4
	C	54	1.39	0.00 (0.0 %)	0.03 (2.2%)	0.00 (0.0%)	0.18 (97.8%)	0.18	13.1
PM6	A	52	1.34	0.01 (1.0 %)	0.00 (0.0%)	0.00 (0.0%)	0.14 (99.0%)	0.14	10.6
	B	54	10.82	0.27 (6.3 %)	0.00 (0.0%)	0.40 (14.4%)	0.95 (79.3%)	1.06	9.8
	C	54	1.37	0.00 (0.0 %)	0.04 (5.1%)	0.00 (0.0%)	0.16 (94.9%)	0.17	12.1
PM7	A	54	54.44	0.00 (0.0 %)	1.97 (3.6%)	0.00 (0.0%)	10.13 (96.4%)	10.32	19.0
	B	54	93.12	0.00 (0.0 %)	0.00 (0.0%)	10.30 (42.3%)	12.02 (57.7%)	15.83	17.0
	C	53	77.58	4.16 (21.7 %)	1.52 (2.9%)	2.12 (5.7%)	7.46 (69.7%)	8.93	11.5

12.8. Precision of Measurement: Multi-Site Reproducibility Study

12.8.1. The purpose of this study was to determine if the IdentiClone Dx *IGH* Assay performs as intended when tested at 3 separate sites. Testing was conducted on a panel containing 6 positive (3 low-positive and 3 mid-positive) and one negative sample with the investigational device in triplicate using 1 reagent lot by 2 operators at 3 different testing sites over 5 different testing instances. The negative panel member was prepared from a pool of clinical specimen gDNA previously determined as Non-Clonal for *IGH*, and the positive panel members consisted of clinical specimen gDNA (previously determined as Clonal for *IGH*) mixed with negative pooled clinical specimen gDNA at 1.5X LOD (low-positive) and 3X LoD (mid-positive).

12.8.2. The negative panel member generated non-clonal results across all three sites in all valid instances (100.0%, 88/88). The low and medium positive panel members generated Clonal results across all three sites in all valid instances (100.0%, 90/90 and 100.0%, 90/90, respectively). Total variation of RPR values due to site, operator, instance, within-run, and the RPR value %CV for each panel member is shown in Table 18 (dominant master mix with highest RPR only for positive PMs, all three master mixes for negative PM).

12.8.3. Overall, the % variance attributed to site had an average of 15.4% and a range of 0.0% - 50.9%.

12.8.3.1. The % variance attributed to operator had an average of 0.5% and a range of 0.0% - 4.3%.

12.8.3.2. The % variance attributed to testing instance had an average of 9.9% and a range of 0.0% - 20.8%.

12.8.3.3. The % variance attributed to within-run had an average of 75.5% and a range of 31.7% - 100.0%, respectively.

Table 18. IdentiClone Dx *IGH* Assay Site-to-Site Precision Variability by RPR value

Control Type	MM	N	Summary Statistics			Variation Type				Total Variation	
			Min	Mean	Max	Between Sites	Between Operators	Between Instances[1]	Within-Run	SD	%CV
Low Positive	A	88	7.00	22.91	29.70	0.00 (0.0 %)	0.04 (0.0%)	0.00 (0.0%)	3.44 (100%)	3.44	15.0
	B	90	15.10	20.16	27.00	0.78 (10.0 %)	0.00 (0.0%)	1.09 (19.6%)	2.07 (70.4%)	2.47	12.2
	C	90	31.10	54.81	72.10	2.40 (10.0 %)	0.43 (0.3%)	3.07 (16.4%)	6.50 (73.3%)	7.59	13.8
Medium Positive	A	90	30.00	48.81	75.60	4.71 (35.1 %)	0.00 (0.0%)	2.13 (7.2%)	6.04 (57.7%)	7.95	16.3
	B	90	26.00	38.83	59.10	2.77 (20.3 %)	0.00 (0.0%)	2.80 (20.8%)	4.72 (58.9%)	6.15	15.8
	C	89	34.30	98.63	141.4	17.36 (50.9 %)	5.06 (4.3%)	8.80 (13.1%)	13.69 (31.7%)	24.32	24.7
Negative	A	88	1.10	1.46	2.00	0.00 (0.0 %)	0.00 (0.0%)	0.07 (12.3%)	0.20 (87.7%)	0.21	14.4
	B	90	1.10	1.43	2.30	0.00 (0.0 %)	0.00 (0.0%)	0.00 (0.0%)	0.21 (100%)	0.21	15.0
	C	90	1.10	1.43	1.90	0.00 (0.0 %)	0.00 (0.0%)	0.00 (0.0%)	0.18 (100%)	0.18	12.5

[1]Testing instances; for each site, there were 5.

12.9. Assay Workflow Validation: DNA Extraction Study

12.9.1. This study provides objective evidence that three common, commercially available genomic DNA (gDNA) extraction methods generate a sufficient gDNA quantity for the IdentiClone Dx *IGH* Assay. Three (3) different commercially available extraction method were evaluated: 1) silica column isolation, 2) magnetic bead-based automated extraction (using the King Fisher Flex), and 3) precipitation were used to extract gDNA from peripheral blood specimen collected in EDTA from 2 unique donors with B-cell lymphoproliferative diagnosis (Clonal) and 8 unique healthy donors (Non-Clonal). Table 19 lists kits by vendor and extraction method.

Table 19: Extraction Kits by Extraction Method

Method	Kit Name	Vendor	Extraction Method
1	QIAamp DNA Blood Mini kit (QIA)	QIAGEN	Silica membrane
2	Promega Wizard® Genomic DNA Purification Kit (PWIZ)	Promega	Precipitation
3	MagMAX DNA Multi-Sample Ultra 2.0 kit (KFF)	Thermo-Fisher Scientific	Magnetic Bead

12.9.2. The samples were extracted using the associated manufacturer instructions, and resulting gDNA was quantified using a UV-VIS Spectrophotometer and NanoDrop 2000. Following quantitation, the gDNA (1 replicate per extraction method) was tested with the IdentiClone Dx *IGH* Assay; completion of data analysis occurred within 48 hours of extraction.

12.9.3. All three extraction methods were shown to generate assay required amount of gDNA, in which every sample generated ≥250ng of gDNA regardless of extraction method. The total gDNA quantity was calculated from the average of 3 replicates of Nanodrop readings for each of the 10 specimens, for each extraction method. (Table 20) Different volumes were used to meet the requirements for each extraction kit:

12.9.3.1. The PWIZ kit required 300 µL peripheral blood and gDNA was eluted in 100 µL, which generated a range of 6,846.7 ng – 26,336.7 ng of total gDNA

12.9.3.2. The QIA kit required 200 µL peripheral blood and gDNA was eluted in 50 µL generated a range of 2,656.7 ng – 10,963.3 ng of total gDNA

12.9.3.3. The KFF kit required 300 µL peripheral blood and gDNA was eluted in 50 µL generated a range of 6,795.0 ng – 33,338.3 ng of total gDNA

Table 20: Total gDNA Extracted Summary

Specimen #	QIA Total gDNA (ng)	QIA average gDNA Concentration (ng/µL)	PWIZ Total gDNA (ng)	PWIZ average gDNA Concentration (ng/µL)	KFF Total gDNA (ng)	KFF average gDNA Concentration (ng/µL)
1	7230.0	144.6	19163.3	191.6	22101.7	442.0
2	3651.7	73.0	7593.3	75.9	10471.7	209.4
3	2656.7	53.1	6846.7	68.5	8313.3	166.3
4	4238.3	84.8	9586.7	95.9	9535.0	190.7
5	4908.3	98.2	13496.7	135.0	14426.7	288.5
6	5496.7	109.9	13506.7	135.1	16171.7	323.4
7	3981.7	79.6	11896.7	119.0	14963.3	299.3
8	4403.3	88.1	11990.0	119.9	6975.0	139.5
9	8776.7	175.5	17043.3	170.4	20606.7	412.1
10	10963.3	219.3	26336.7	263.4	33338.3	666.8

12.9.4. All 30 replicates (10 specimens tested with 3 extraction methods) generated at least 250 ng of gDNA (30/30, with lower limit of the 95% Confidence Interval ≥80%). The total gDNA recovered from the PWIZ and KFF methods were similar because the same peripheral blood input volume (300 µL) per extraction was used, as compared to QIA, that used a lower peripheral blood input volume (200 µL). When tested with the IdentiClone Dx *IGH* Assay, the extracted gDNA exhibited similar RPR values (%CV ranging from 1% - 21%) and concordant master mix results across all three extraction methods. (Table 21) Sample processing (including DNA extraction) to result (including data analysis) took 28 hours or less for all samples.

Table 21: Summary of IdentiClone Dx *IGH* Assay results from the 3 extraction methods

Sample	MM or Overall	QIA RPR	PWIZ RPR	KFF RPR	%CV
PM1	A	1.5	1.5	1.3	7%
	B	1.6	1.8	1.2	16%
	C	1.4	1.3	1.4	3%
	Overall	**Non-Clonal**	**Non-Clonal**	**Non-Clonal**	**N/A**
PM2	A	SQ error*	1.4	1.3	4%
	B	1.3	1.3	1.5	7%
	C	1.8	1.1	1.4	20%
	Overall	**N/A**	**Non-Clonal**	**Non-Clonal**	**N/A**
PM3	A	1.3	1.6	1.5	9%
	B	1.5	1.1	1.6	15%
	C	1.8	1.2	1.2	20%
	Overall	**Non-Clonal**	**Non-Clonal**	**Non-Clonal**	**N/A**
PM4	A	Invalid	1.2	1.4	8%
	B	1.8	1.4	1.4	12%
	C	1.2	1.4	1.3	6%
	Overall	**N/A**	**Non-Clonal**	**Non-Clonal**	**N/A**
PM5	A	1.5	1.4	1.2	9%
	B	1.4	1.1	1.5	13%
	C	1.2	1.2	1.1	4%
	Overall	**Non-Clonal**	**Non-Clonal**	**Non-Clonal**	**N/A**
PM6	A	1.6	1.2	1.2	14%
	B	1.2	1.5	Invalid	11%
	C	1.4	1.6	1.6	6%
	Overall	**Non-Clonal**	**Non-Clonal**	**N/A**	**N/A**

Table 21: Summary of IdentiClone Dx *IGH* Assay results from the 3 extraction methods

Sample	MM or Overall	QIA RPR	PWIZ RPR	KFF RPR	%CV
PM7	A	1.2	1.3	1.6	12%
	B	1.5	1.7	1.3	11%
	C	1.7	1.4	1.2	14%
	Overall	**Non-Clonal**	**Non-Clonal**	**Non-Clonal**	**N/A**
PM8	A	1.3	1.2	Invalid	4%
	B	1.5	1.7	1.5	6%
	C	1.3	1.3	1.6	10%
	Overall	**Non-Clonal**	**Non-Clonal**	**N/A**	**N/A**
PM9	A	1.7	1.3	1.6	11%
	B	66.3	112.8	86.9	21%
	C	76.5	65.9	71.5	6%
	Overall	**Clonal**	**Clonal**	**Clonal**	**N/A**
PM10	A	145.9	185.7	165.9	10%
	B	196.7	192.6	196.9	1%
	C	233.2	199.1	216.5	6%
	Overall	**Clonal**	**Clonal**	**Clonal**	**N/A**

*Was not retested.
N/A = Not Applicable

12.10. Assay Workflow Validation: DNA Input Study

12.10.1. The purpose of this study is to provide objective evidence of the gDNA input requirements for the IdentiClone Dx *IGH* Assay. Evaluation of 5 different gDNA input quantities (500 ng, 250 ng, 200 ng, 125 ng and 25 ng) was performed on 10 clinical samples (5 with a positive clinical diagnosis and 5 with a negative clinical diagnosis of B-cell Lymphoproliferation) using the IdentiClone Dx *IGH* Assay. The concentration of all gDNA samples was measured using the same Nanodrop 2000 Spectrophotometer. Testing was performed in duplicate over 6 runs, on 1 ABI 3500xL Dx instrument, by 1 operator using 1 reagent lot, over multiple days.

12.10.2. Of the 100 possible clonality status results (10 specimens tested in duplicate for 5 different input conditions), 96 overall results (using all 3 master mixes). The 4 samples that failed to generate overall results contained the lowest DNA input level (25 ng), in which replicates had invalid results due to low amplification in one or more master mixes. The likely cause of these invalid results is insufficient DNA input therefore, these invalids were not re-tested. Of the 96 overall results generated, 95 were concordant with the expected results; the overall concordance is 99.0%; Table 22 provides the clonality status result concordance per panel member and DNA input level. The 1 discordant result occurred in PM09, which contained the lowest DNA input level (25 ng); one replicate of this expected Non-Clonal panel member generated a "Clonal" result with master mix B, generating an RPR value of 3.2, which is above the assay cut-off of 3.0. This discordant result was potentially due to the low DNA input, which may have reduced the background peak RFU (denominator for the RPR calculation) more than the RFU of the highest peak (numerator for the RPR calculation), thus artificially inflating RPR to be slightly above the cut-off RPR of 3.0. Of the 20 clonality status results generated with the intended DNA input (250 ng), all were concordant with the expected results. The overall result concordance for the intended DNA input level is 100.0%.

Table 22: Overall Result Concordance

Panel Member	Expected Clonality	Overall Result Concordance				
		500 ng	250 ng (Intended)	200 ng	125 ng	25 ng
PM01	Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)
PM02	Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)
PM03	Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)
PM04	Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)
PM05	Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)

Table 22: **Overall Result Concordance**

Panel Member	Expected Clonality	Overall Result Concordance				
		500 ng	250 ng (Intended)	200 ng	125 ng	25 ng
PM06	Non-Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	N/A
PM07	Non-Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	N/A
PM08	Non-Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)
PM09	Non-Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	50% (1/2)
PM10	Non-Clonal	100% (2/2)	***100% (2/2)***	100% (2/2)	100% (2/2)	100% (2/2)
Overall		**100% (20/20)**	**100% (20/20)**	**100% (20/20)**	**100% (20/20)**	**93.8% (15/16)**

12.11. Assay Workflow Validation: Equivalency:3500xL vs 3500xL Dx

12.11.1. This study demonstrates that the IdentiClone Dx *IGH* Assay results are equivalent when obtained using two different versions of a similar instrument:

12.11.1.1. The Applied Biosystems 3500xL Genetic Analyzer (RUO), and

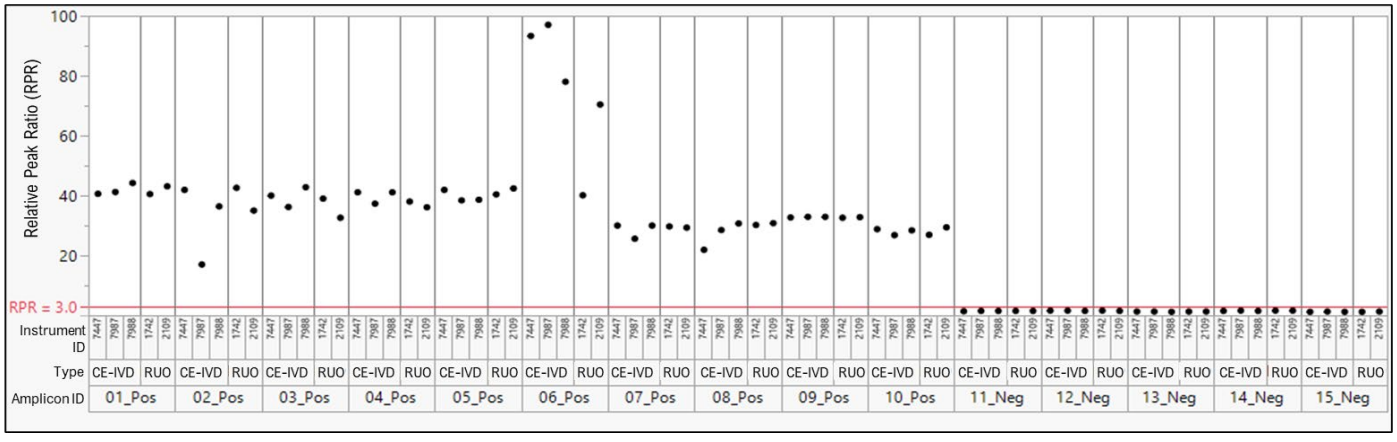
12.11.1.2. The Applied Biosystems 3500xL Dx Genetic Analyzer (CE-IVD)

12.11.2. Samples comprised of 15 amplicons from 10 Clonal and 5 Non-Clonal samples (in addition to the amplicons of the associated run controls) and were tested with the IdentiClone Dx *IGH* Assay on 5 different ABI 3500 instruments (2 RUO and 3 CE-IVD) each.

12.11.3. A total of 75 valid results were generated by the 5 different instruments (15 amplicons x 1 replicate x 5 instruments). These results were further evaluated to calculate the relative peak ratio (RPR) values, clonality status results, average RPR by instrument type, and average RPR difference between instrument types. (Figure 12)

12.11.4. Test results generated with the 15 amplicons (10 Clonal/positive and 5 Non-Clonal/negative) were 100% concordant with the expected results across all 5 instruments. The RPR values generated by the positive amplicons ranged from 17.0 - 97.0, and the RPR values generated by the negative amplicons ranged from 1.2 - 1.6. A maximum of 61.9% difference was calculated from average RPR values generated by the different instruments; however, this can be attributed to one "outlier" RUO test result, which generated significantly lower RPR value, as compared to the results of the other four instruments (40.1, compared to 70.4, 78.0, 93.3, 97.0 and 78.0, respectively). Since this amplicon is a high-positive (high % clonality), it is unlikely to cause a difference in the clonal status result when tested on one instrument type versus the other. The highest percent difference observed for the remaining positive and negative amplicons were 18.1% and 4.2%, respectively. Overall, the 100.0% concordant results produced from the 5 different instruments demonstrate that the performance of the IdentiClone Dx *IGH* Assay on ABI 3500xL Dx and ABI 3500xL Genetic Analyzers are equivalent.

Figure 12: **RPR values generated by each amplicon, per instrument type (CE-IVD vs. RUO), per instrument ID**



12.12. Clinical Validation

12.12.1. Pivotal Clinical Accuracy Study Overview

12.12.1.1. This pivotal accuracy study was designed to evaluate the clinical performance equivalency between the IdentiClone Dx *IGH* Assay (the investigational device) and a predicate device on retrospective and residual de-identified DNA extracted from peripheral blood (PB) samples from individuals with suspected B-Cell Lymphoproliferations. The predicate device is a commercially available IVD assay (at the time of the study) that applies NGS to detect clonal *IGH* gene rearrangements (Reference Assay), with an intended use similar to the investigational device, the IdentiClone Dx *IGH* Assay on the same sample type.

12.12.1.2. The IdentiClone Dx *IGH* Assay has been developed by Invivoscribe as an *in vitro* diagnostic device intended for qualitative capillary electrophoresis-based detection of clonal immunoglobulin heavy chain (*IGH*) gene rearrangements in genomic DNA extracted from peripheral blood of patients with suspect lymphoproliferations. The Reference Assay uses NGS technology to identify clonal *IGH* V_H - J_H rearrangements, the associated V_H - J_H region DNA sequences and the frequency distribution of V_H and J_H region segment utilization.

12.12.2. Study Objectives

12.12.2.1. The objectives of this pivotal accuracy study were to 1) establish agreement between the results of the IdentiClone Dx *IGH* Assay and the Reference Assay by assessing the positive and negative percentage agreement between the two assays and 2) estimate relationship between disease diagnoses and clonality detection.

12.12.3. Patient Population

12.12.3.1. A total of 303 samples were enrolled in the study; however, 54 samples were not eligible for comparison between the Reference assay and the Investigative device (IdentiClone Dx *IGH* Assay). Of these, 4 samples were not shipped to the testing site, and 50 samples were either Quantity Not Sufficient (QNS) or had results that were not evaluable with at least one assay, rendering them unsuitable for inclusion in the comparative analysis. The remaining 249 samples comprised of two analysis populations, 1) an analysis set *with* healthy subjects (n=249), which included DNA from peripheral blood of subjects suspected of lymphoproliferative disease with negative B-cell clonality results or DNA extracted from normal, healthy peripheral blood specimens and 2) an analysis set *without* healthy subjects (n=203), which included DNA samples from patients with B-cell lymphoproliferative disease diagnosis.

12.12.3.1.1. All subjects (samples) enrolled in the study were included in the Enrollment Set (ES) . The agreement analysis between the IdentiClone Dx *IGH* Assay and the Reference Assay used the ES population.

12.12.3.1.2. The Primary Analysis Set (PAS) includes samples from the population with positive, negative or invalid/indeterminate results from the IdentiClone Dx *IGH* Assay results and positive or negative results from the reference method.

12.12.4. Selection of Patients and Aliquots for IdentiClone Dx *IGH* Assay

12.12.4.1. The study design utilized de-identified leftover DNA samples from clinical testing and, therefore, was exempt from requiring individual subject consents.

12.12.4.2. Only one sample per subject was included in the final analysis set used to assess primary objectives.

12.12.5. Safety Analysis

12.12.5.1. The IdentiClone Dx *IGH* Assay is not expected to pose direct risk to subject safety and no treatment decisions were made based on the IdentiClone Dx *IGH* Assay results.

12.12.6. Effectiveness

12.12.6.1. Primary IdentiClone Dx *IGH* Assay Clinical Validation

12.12.6.1.1. The primary intent of this clinical study establishes agreement between the Investigational Device (IdentiClone Dx *IGH* Assay) and a Reference Assay (a commercially available IVD assay [at the time of the study] that applies NGS to detect clonal IGH gene rearrangements, with an intended use similar to the investigational device).

12.12.6.1.2. Concordance between the Investigational device (IdentiClone Dx *IGH* Assay) and the Reference Assay clonality results are displayed in Table 23. Among the 249 evaluable samples evaluable by the reference method, 243 reported the same result by both methods.

Table 23: **Concordance Between IdentiClone Dx *IGH* Assay (IUO) and Reference Assay (IVD) Including Healthy Subjects**

IdentiClone Dx *IGH* Assay (IUO)	Reference Assay (IVD)		
	+	-	Total
+	129	1	***130***
-	5	114	***119***
Total	***134***	***115***	**249**

12.12.6.1.3. Table 24 details concordance based on agreement between clonality results from the IdentiClone Dx *IGH* Assay (IUO) and the NGS -based Reference Assay (IVD). Among the 249 evaluable samples tested, 114 of the 115 negative (-) samples were concordant between both methods, 129 of 134 of the positive (+) samples were concordant, for NPA of 99.1% and PPA of 96.3%. The lower limit of the 95% confidence interval (CI) for the NPA is 95.3% while the lower limit of the 95% CI for the PPA is 91.5%; Table 24 details the agreement between the Investigational Device and the Reference Method. The positive and negative likelihood ratios are provided in Table 25.

Table 24: **Agreement Between the Investigational Device and Reference Assay**

Measure of Agreement	Percent Agreement (n/N)	95% CI[1]
NPA	99.1% (114 / 115)	(95.3%, 100.0%)
PPA	96.3% (129 / 134)	(91.5%, 98.8%)

[1]The 95% CI is calculated using the Exact (Clopper-Pearson) method

Table 25: **Likelihood Ratio Between IdentiClone Dx *IGH* Assay and Reference Assay (IVD) Including Healthy Subjects**

Positive Likelihood Ratio	Negative Likelihood Ratio
110.709	0.038

12.12.6.1.4. Table 26 presents concordance between the Investigational Device and the Reference Assay clonality results *without* healthy subjects. Among the 203 evaluable samples evaluable by the reference method, 197 reported the same result by both methods.

Table 26: **Concordance Between IdentiClone Dx *IGH* Assay (IUO) and Reference Assay (IVD) Excluding Healthy Subjects**

IdentiClone Dx *IGH* Assay (IUO)	NGS-based Reference Assay (IVD)		
	+	-	Total
+	128	1	***129***
-	5	69	***74***
Total	***133***	***70***	**203**

12.12.6.1.1. Concordance is based on agreement between the Investigational Device and the Reference Assay *excluding* healthy subjects (Table 27). Among the 203 evaluable samples tested, 69 of the 70 negative (-) samples were concordant between both methods, 128 of 134 of the positive (+) samples were concordant, for NPA of 98.6% and PPA of 96.2%. The lower limit of the 95% CI for the NPA is 92.3% while the lower limit of the 95% CI for the PPA is 91.4%. The positive and negative likelihood ratios are provided in Table 28.

Table 27: **Agreement Between IdentiClone Dx *IGH* Assay (IUO) and Reference Assay (IVD) Excluding Healthy Subjects**

Measure of Agreement	Percent Agreement (n/N)	95% CI[1]
NPA	98.6% (69/ 70)	(92.3%, 100.0%)
PPA	96.2% (128/ 133)	(91.4%, 98.8%)

[1]The 95% CI is calculated using the Exact (Clopper-Pearson) method

Table 28: Likelihood Ratio Between IdentiClone Dx *IGH* Assay and Reference Assay (IVD) Excluding Healthy Subjects

Positive Likelihood Ratio	Negative Likelihood Ratio
67.368	0.038

12.12.7. Conclusions

12.12.7.1. This clinical evaluation of the IdentiClone Dx *IGH* Assay confirms that the investigational device accurately detects clonal events in the immunoglobulin heavy chain (*IGH*) gene.

12.12.7.2. This pivotal accuracy study provides documented evidence that the investigational device and the reference method (predicate device) have similar detection rates for *IGH* clonal events. Agreement primary objectives were set at 70% of a lower limit of a 95% CI for both NPA and PPA since these two technologies are quite different. However, the trial results prove that these two technologies identify clonal events at a very similar rate.

12.12.7.3. Residual de-identified DNA extracted from peripheral blood (PB) samples from individuals with suspected B-cell lymphoproliferative disorders were tested by both methods. A subset of 249 samples generated evaluable results with both assays and the study acceptance criterion was met:

12.12.7.3.1. The lower limit of the NPA 95% exact CI needed to be greater than or equal to 70%. The lower limit achieved was 95%.

12.12.7.3.1.1. Diagnostic Specificity was calculated to be 0.991.

12.12.7.3.1.2. Negative Likelihood Ratio: 0.038

12.12.7.3.2. The lower limit of the PPA 95% exact CI was greater than or equal to 70%. The lower limit achieved was 92%.

12.12.7.3.2.1. Diagnostic Sensitivity as calculated to be 0.963.

12.12.7.3.2.2. Positive Likelihood Ratio: 110.709

12.12.7.4. There was one false positive and five false negative results with the investigational method.

12.12.7.5. This clinical evaluation of the IdentiClone Dx *IGH* Assay confirms that the investigational device accurately detects clonal events in the *IGH* gene.

12.12.7.6. No safety concerns were identified during the execution of this study.

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- IdentiClone Dx *IGH* Software Instructions for Use – English (Invivoscribe 📖: 310000)
- ABI 3500xL Dx Genetic Analyzer User Manual (Thermo Fisher: 100079380 Revision D)
- ABI 3500xL Genetic Analyzer User Manual (Thermo Fisher: 4401661 Revision C)

14. Technical and Customer Service

Technical and Customer Service Representatives are available Monday through Friday to answer phone, e-mail, or website inquiries.

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15. Symbols

The following symbols are used in labeling for this product.

REF	Catalog Number	TAQ	Taq DNA Polymerase
VOL	Reagent Volume	CONTROL + *IGH*	*IGH* Positive Control
LOT	Lot Number	CONTROL - *IGH*	*IGH* Negative Control
	Storage Conditions	CONTROL NTC	No Template Control (NTC)
UDI	Unique Device Identifier	MMA *IGH* FR1	MMA *IGH* FR1 (Master Mix A)
	Expiration Date	MMB *IGH* FR2	MMB *IGH* FR2 (Master Mix B)
EC REP	Authorized Representative in the European Community	MMC *IGH* FR3	MMC *IGH* FR3 (Master Mix C)
CE	European Conformity		Consult Instructions for Use
IVD	For *In Vitro* Diagnostic Use	CH REP	Swiss Authorized Representative

16. Legal Notice

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