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VIGILANTBIOSCIENCES®

Ora-3D

Instructions for Use

For additional product information, visit www.vigilantbiosciences.com

Users must read this package insert in its entirety before using the product. Follow the instructions carefully when conducting the test. Failure to do so may cause inaccurate test results.

PRODUCT NAME AND INTENDED USE

The Ora-3D is a point-of-care semi-quantitative immunoassay consisting of a single use lateral flow test that measures p16 and EGFR in saliva, which are known to be associated with oral cancer. The Ora-3D is intended to be used by qualified health-care providers as a pre-diagnostic test on symptomatic adult patients, specifically those with oral mucosal abnormalities visible to the health-care provider, during the oral cancer exam and risk assessment. The Ora-3D is not automated and requires qualified health-care provider to administer and recommend next steps. The Ora-3D is intended to provide additional objective information that can support clinician's decision to refer a patient for further evaluation as needed. It does not replace the standard of care. The Ora-3D is intended to be used with the BeVigilant™ Reader. The BeVigilnt™ Reader provides the summary of the combined test result with clinical risk factors to assess oral abnormalities in patients with lesions. The Ora-3D is not a standalone diagnostic test and is not intended for diagnosis.

For professional use only.

For in *vitro* diagnostic use only.

PRINCIPLES OF OPERATION

The Ora-3D is a semi-quantitative point of care device consisting of a single use collection device and a single-use lateral flow immunoassay that measures p16 and EGFR in saliva, which are known to be associated with oral cancer. The BeVigilant™ Reader is the electronic enclosure with integrated electronics and software used to input clinical risk factor and report the result of the test. The clinician initiates a test by selecting 'New Test' on the BeVigilant™ Reader and entering patient data and clinical factors. The clinician then collects a saliva sample from the patient using the Saletto™ Oral Fluid Collection Device (Image 3), which filters the sample. As a last step, the clinician applies it to the specified wells on the test cassette. The cassette is then placed in the positioning tray of the BeVigilant™ Reader (Image 1) and the user reviews the OraFusion Software and taps 'Start Test.' The BeVigilant™ Reader analyzes the sample and provides a test result. The BeVigilant™ Reader is not automated and requires the health-care provider to administer the test and recommend next steps .

MATERIALS PROVIDED IN THE BEVIGILANT™ READER TO ADMINISTER A TEST

Component Description	Quantity
BeVigilant™ Reader	1
Power Adapter & Interchangeable Blade Kit	1
Instructions for Use (IFU)	1
Quick Reference Guide (QRG)	1

Image 1-BeVigilant™ Reader

MATERIALS PROVIDED IN THE ORA-3D TO ADMINISTER A TEST

Component Description	Quantity
Ora-3D Pouch	12
Oral Fluid Collection Device	12
Instructions for Use (IFU)	1
Quick Reference Guide (QRG)	1

Image 2 – Ora-3D Cassette

Image 3 – Porex Saletto™ Oral Fluid Collection Device

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ORA-3D & BEVIGILANT™ READER WARNINGS AND PRECAUTIONS

- Failure to follow the instructions provided may lead to inaccurate results.
- This test is not intended for diagnosis.
- This test is intended for use by health-care providers in a health-care setting.
- This test should only be used for symptomatic adult patients with oral mucosal abnormalities visible to the health-care provider during the oral cancer exam.
- This test has been developed for use with saliva only. The use of this test with any other specimen type may lead to inaccurate results.
- The use of a saliva specimen with blood, food, alcohol or any other substances present in the oral cavity within 1 hour may lead to false negative/positive results.
- Do not use this device if there is an infection or fungus in the mouth, blood present in the saliva sample, or the patient has an active cancer diagnosis.
- Saliva sample should be used immediately after collection.
- Wear appropriate personal protective equipment and use standard office practices when handling and testing the patient specimen.
- Use the Ora-3D immediately after opening the pouch.
- Stop using immediately if an allergic reaction occurs.
- Insufficient saliva sample volume may lead to inaccurate results.
- This product has not been tested on pregnant women. The OraFusion System has only been tested on adults of age 22 years or older.
- This test is intended to be performed at room temperature (16°C to 30°C); do not use out of this range.
- The recommended storage temperature for the test kit is 4°C to 40°C.
- Use all test devices only once and dispose properly. Do not reuse any of the test devices.
- The BeVigilant™ Reader is not intended to be moved during a test.
- Do not use Ora-3D after the expiration date on the package. Expired devices should be disposed of according to local, state, and federal waste disposal requirements.
- Used Ora-3D are considered a potential biohazard and should be disposed of according to local, state, and federal waste disposal requirements.

- Do not position the equipment in a way that would make it difficult to reach the external power supply.
- Not properly plugging the charging cable into the BeVigilant™ Reader may cause the battery to lose charge and Reader to not power on.
- Ensure that the Reader is plugged into the appropriate power source.
- Do not expose ports to liquids; this can cause a short circuit and overheating.
- Maintain the environmental conditions explained in Safety Warnings for proper equipment operation.
- Modification of this device is not allowed.
- Ora-3D does not have backward compatibility.
- Use only with recommended consumables, accessories, or medical devices.
- Examine detachable power cord monthly for continued safe usage.
- The BeVigilant™ Reader may be reused until signs of material degradation occur. Do not use the BeVigilant™ Reader if the device shows signs of ageing, wear, fatigue, or any degradation as suggested by changes in its appearance that may affect performance.
- Install software updates immediately upon notification.
- Ensure the BeVigilant™ Reader is calibrated prior to use.
- Do not use Ora-3D if components are damaged or missing.
- Do not use the BeVigilant™ Reader if unresponsive or components are damaged or missing.
- Discontinue device use in the event of a malfunction.
- Label samples prior to testing to prevent mix-ups.
- Do not write over or damage the QR Code. If damage occurs, the cassette will return an invalid test result.
- Natural rubber latex was not used as a material in the manufacture of a medical product, its container and/or packaging.
- Avoid distractions while administering the test. The patient should remove any lipstick or lip treatment such as lip balm, ointment etc. prior to specimen collection for the administration of the test.

ORA-3D CONTRAINDICATIONS

There are no contraindications for the Ora-3D.

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ORA-3D ENVIRONMENTAL CONDITIONS

The permissible environmental conditions of use for proper operation of the Ora-3D are:

Operational temperature:	16°C to 30°C
Operational Humidity:	10% to 85% relative humidity, non-condensing
Storage Temperature:	4°C to 40°C

BEVIGILANT™ READER PROCEDURE FOR INITIAL SET UP

1. Remove the BeVigilant™ Reader from the box.
2. Place the BeVigilant™ Reader on a flat open surface near a power outlet.
3. Remove the power adapter & Interchangeable Blade Kit from the box.
4. If needed, replace the interchangeable blade on the power adapter by pressing down on the spring-loaded tab, insert the blade adapter at 60° where the top is flat, and the bottom has a U-shape. Press the adapter down towards the power supply until it locks in place, a clicking sound will occur. if more information is needed, please visit: http://www.globtek.com/pdf/Instructions-Interchangeable-Blades.pdf
5. Plug into a power outlet.
6. Plug the power cord into the reader ensuring it is connected securely.
7. Press the power button, located on the back of the device. The power button will illuminate when device is on.
8. Ensure the BeVigilant™ Reader is powered on (if the screen does not light up after the power button is activated, check all connections and ensure the outlet being used is functioning properly).
9. Select the language to be used on the device.
10. Select the Time zone where the device is used.
11. Review the Privacy Policy and tap Accept. Tap CONTINUE to proceed.
12. Review the Terms of Use and tap Accept. Tap CONTINUE to proceed.
13. Connect the reader to a wireless internet network by selecting the network, entering the appropriate network password, and tap CONNECT.
14. Create an account for the practice by entering the Practice Name, Email Address, and Password (enter the password twice for confirmation) and tap CREATE ACCOUNT.

15. An automated message will be sent to the email address used to create the account. Check the email (if it is not in the inbox, check any spam filters) to get the Verification Code.
16. Enter the verification code in the reader. NOTE: Verification code is valid for a limited time.
17. If correct verification code is entered, the reader will prompt to create a 4-digit App PIN.
18. Create a 4-digit App PIN for the app to expedite future log in.
19. Re-enter the 4-Digit App PIN to confirm.
20. The reader is now ready to perform the Ora-3D. Set up is complete.

PLEASE NOTE: The USB is for Vigilant use in regard to internal testing and/or accessing data- each Reader is password protected in order to prevent unauthorized access.

BEVIGILANT™ READER PROCEDURE FOR USE

Perform the following steps to initiate a new Ora-3D test:

1. Place the BeVigilant™ Reader on a flat open surface where the test will be performed. The reader should not be moved during testing. Movement may invalidate the test.
2. Tap the reader screen to ensure it is powered on. If the screen doesn't respond, press the power button on the back of the device for 1 second.
3. Enter the App PIN to log into the app.
4. Tap START NEW TEST, then enter Year of Birth and Gender. Record the Test ID for traceability between the Ora-3D test results and the practice's Quality system for future reference.
5. Enter the patient Clinical Risk Factors by tapping the appropriate answer for each factor. (For additional information about Clinical Risk Factors, tap the ⓘ.
6. Enter if the patient has fasted from food or fluid within 1 hour of the test. If yes, continue; If no, enter if the patient has had any of the cross-reactivity substances listed.
7. Prepare a saliva sample for testing by following the Specimen Collection Procedure for Use next section. After collecting the sample, the BeVigilant™ OraFusion Software will act as a guide through the test steps.

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SPECIMEN COLLECTION PROCEDURE FOR USE

Perform the following steps to prepare a patient sample for Ora-3D.

- Prior to collecting a sample, follow the BeVigilant™ Reader Procedure For Use.
- Gloves must be worn by the clinician when performing the Ora-3D.

The OraFusion Software will guide user through the following steps.

- Remove a Collection Device and a Test Cassette from the box. Inspect the packaging for damage and do not use items past the expiration date printed on the pouch. Only open the components as needed.

Important! Patients should not eat, drink, chew gum, smoke, gargle mouthwash, or put anything in or around the mouth for at least 1 hour before sample collection. Substances in the mouth may cause false test results.

IMPORTANT FOR SALIVA TRANSFER:

1. **Place the sponge only on the top of the tongue. DO NOT place the sponge under the tongue.**
2. **Ensure to POOL saliva prior to placing the sponge in the mouth.**
3. **Maintain the tongue in one position while the sponge absorbs saliva.**
4. **DO NOT perform any sucking motion while the sponge is absorbing saliva.**
5. **DO NOT place your mouth over the plastic body of the collection device.**

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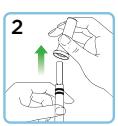
1. Instruct the patient to pool saliva in the mouth for a minimum of 30 seconds.
2. Remove the Push Cap and set aside.
3. Instruct the patient to place the entire Sponge in the mouth and saturate with saliva for a minimum of 90 seconds. Continue to pool and saturate the sponge in the mouth during the 90 seconds.
4. After 90 seconds, check if the indicator has turned solid blue. If the indicator has not turned blue (indicating full saturation), repeat steps 1 through 3.
5. Retrieve the properly filled collection device from the patient. Place the Collection Device cap over the Sponge.
6. Keeping the device upright, push the cap down slowly and smoothly in one motion to filter the saliva into the sample dropper.
7. Saliva should be present in the bottom of the dropper tip. Ensure the minimum volume is up to the top of the purple dropper tip. If there is not enough, repeat steps 1 through 6. The sample is ready for testing. Proceed to the Ora-3D & BeVigilant™ Reader procedure for use.
8. Inspect the filtered saliva in the tube for blood. Select the option on the OraFusion Software to continue.
9. Perform test within 1 hour of saliva collection.

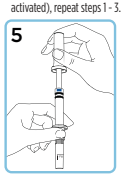
Pool saliva in mouth for 30 seconds.



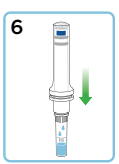
Remove Push Cap and set aside.

Put entire Sponge in mouth and saturate with saliva for 90 seconds. Do not chew or squeeze Sponge.

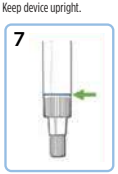
Indicator turns solid BLUE when saturated with saliva.
Remove Sponge from mouth to check Indicator. If it is not solid Blue (fully activated), repeat steps 1 - 3.



Replace Push Cap over Sponge.
Keep device upright.



Keeping device upright, push Push Cap down slowly and smoothly, in one motion.
Compress Sponge completely.



Minimum volume of Saliva shall be to top of the dropper

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ORA-3D & BEVIGILANT™ READER PROCEDURE FOR USE

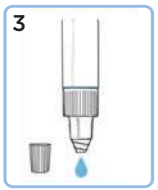
1. Prior to administering a test, follow the BeVigilant™ Reader Procedure For Use and Specimen Collection Procedure For Use.
2. Remove the Ora-3D Test cassette from the packaging and place on flat surface near the BeVigilant™ Reader and prepare to apply the filtered saliva to the cassette.
3. Unscrew the dropper cap from the sample dropper nozzle.

Caution: Some saliva may flow without pressing the dropper tube. Ensure cleaning supplies are available if needed.

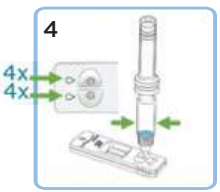
4. Add four drops of the sample into each well of the test cassette (eight drops in total) by squeezing the tube. The sample may pool in the well when first applied. Allow time for the saliva to saturate into the cassette before moving the cassette into the positioning tray.

Important! Do not exceed a 60 second timeframe between dropping the sample into the cassette and beginning the test.

5. Place the test cassette into the positioning tray
6. Confirm all previous steps have been completed.
7. Tap START TEST on the BeVigilant™ Reader screen. If the elapsed time is greater than 1 hour since the lapse timer started, the sample has expired, and an error message will occur.
8. When the Sensor Fusion is complete, the BeVigilant™ Reader screen will display a Total Risk Profile result showing LOW RISK, MODERATE RISK, or ELEVATED RISK of oral cancer, based on the analysis of specific biomarkers and individual Clinical Risk Factors.



Remove the dropper cap and set aside



Add a four (4) drops of the sample into each well of the test cassette by squeezing the tube.

9. Tap DONE to return to the home screen.
10. Remove the test cassette from the BeVigilant™ Reader and dispose of the cassette and Collection Device according to local, state, and federal waste disposal requirements.

If an error occurs, the screen will display an alert. A new test can be immediately administered using a new Ora-3D.

If you wish to view the test again, it can be displayed through the home screen by selecting "FIND TEST" and entering the Test ID previous recorded in the practice's Quality system.

INTERNAL QUALITY CONTROL

A control line is built into each Ora-3D, demonstrating the sample is properly flowing through the test. If the control line is not detected by the reader, the BeVigilant™ Reader will display an error message. A new test should be performed as explained above.

QUALITY CONTROL PROCEDURE

- Inspect the expiration date on the package for the Ora-3D to ensure the product is not expired.
- Examine detachable power cord monthly for continued safe usage.
- Ensure the most recent software is installed prior to use if the device has been unused for an extended period of time.

DISPOSAL

Used Ora-3D are considered a potential biohazard and should be disposed of according to local, state, and federal waste disposal requirements. Ensure that the BeVigilant™ Reader is handled in accordance with WEEE and Batteries Directive.

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ANALYTICAL PERFORMANCE CHARACTERISTICS OF ORA-3D

The laboratory performance results of the Ora-3D are shown in Table 1 below.

Table 1. Analytical Performance Characteristics*

Analytical Performance Characteristic	p16 Result	EGFR Result
Analytical Sensitivity	1.5 ng/ml (a)	1 ng/ml (a)
Analytical Specificity	>95%	>95%
Trueness (Bias)	±20% (approximation)	±20% (approximation)
Precision (Repeatability & Reproducibility)	±20% (approximation)	±20% (approximation)
Accuracy (Results from Trueness & Precision in artificial saliva)	80% (c)	80% (c)
Limit of Detection and Quantitation	1.5 ng/ml (a)	1 ng/ml (a)
Relevant Interferences	Candida albicans	Human Serum Albumin
Cross-Reactions	Mouthwash	Human Serum Albumin
Limitations of Method	Saliva stability, sample delivery, chemical purity	Saliva stability, sample delivery, chemical purity
Measuring Range	1.5 – 96 ng/ml	1.0 – 16 ng/ml
Cut-Off	15 ng/ml (d)	0.9 ng/ml (d)
Linearity	R2 = 0.9959 (e)	R2 = 0.9997 (e)
Available Reference Measurement Procedures	Enzyme-linked immunoassay (ELISA) test	Enzyme-linked immunoassay (ELISA) test

*Results based on artificial saliva (Synthetic Oral Fluid, UTAK, PN 35400-SMX-OF(F); Exp: 31JAN2024 C9465). These will be updated using whole human saliva in a new revision of the document.

a. Specific sensitivity and Limit of Detection are defined in EP17-2A as being the same value.
b. The trueness and precision values shall be determined from diluted filtered whole human saliva into buffer The measuring range was determined from artificial saliva samples used to build a dose – response curve in the physiological range of the biomarker.
c. The accuracy value was calculated by subtracting the precision from 100% to give a relative value for accuracy.
d. The cut-off values were estimated from the reference measurement method, ELISA using filtered whole human saliva.
e. These values were derived from the linear regression of the data obtained for dose response curves for EGFR and p16.

CLINICAL PERFORMANCE CHARACTERISTICS OF ORA-3D+

The clinical performance characteristics of the Ora-3D++ are shown in Table 2 below.

Table 2. Clinical Performance Characteristics

Clinical Performance Characteristic	p16 Result	EGFR Result
Threshold Value	15ng/ml (a)	0.9ng/ml (a)
Diagnostic Sensitivity	88% (a)	92% (a)
Diagnostic Specificity	92% (a)	100% (a)
Positive Predictive Value	90% (a)	100% (a)
Negative Predictive Value	91% (a)	94% (a)
Likelihood Ratio	12 (b)	Infinity (b)
Expected Values in Normal Populations	7.7 ng/ml (a)	0.2 ng/ml (a)
Expected Values in Affected Populations	60.9 ng/ml (a)	3.6 ng/ml (a)

+ These are ex-vivo, and known prevalence based whole human saliva samples.
++ These results do not include reader components, only the Ora-3D cassettes in this revision.
a. These values were determined by the enzyme-linked immunoassay testing of a positive cohort (donors with oral cancer) and negative cohort (donors with no oral cancer).
b. Calculated as the positive likelihood ratio (diagnostic sensitivity/ (1-diagnostic specificity)). Threshold values are cut-off values.

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GLOSSARY OF SYMBOLS

Standard/ Source	Symbol	ISO/IEC symbol number	Title of Symbol	Description of Symbol Per Standard
ISO 15223-1:2021 5.1.1		ISO 7000-3082	Manufacturer	Indicates the medical device manufacturer
ISO 15223-1:2021 5.1.2		N/A	Authorized representative in the European Community/ European Union manufacturer	Indicates the authorized representative in the European Community/ European Union
ISO 15223-1:2021 5.1.3		ISO 7000-2497	Date of Manufacture	Indicates the date when the medical device was manufactured
ISO 15223-1:2021 5.1.4		ISO-7000-2607	Use-by-date	Indicates the date after which the medical device is not to be used.
ISO 15223-1:2021 5.1.5		ISO 7000-2492	Batch Code	Indicates the manufacturer's bath code so that the batch or lot can be identified.
ISO 15223-1:2021 5.1.6		ISO 7000-2493	Catalog number	Indicates the manufacturer's catalogue number so that the medical device can be identified.
ISO 15223-1:2021 5.1.7		ISO 7000-2498	Serial Number	Indicates the manufacturer's serial number so that a specific medical device can be identified.
ISO 15223-1:2021 5.3.4		ISO 7000-0626	Keep Dry	Indicates a medical device that needs to be protected from moisture.

Standard/ Source	Symbol	ISO/IEC symbol number	Title of Symbol	Description of Symbol Per Standard
ISO 15223-1:2021 5.3.7		ISO 7000-0632	Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed.
ISO 15223-1:2021 5.3.8		ISO 7000-2620	Humidity limitation	Indicates the range of humidity to which the medical device can be safely exposed.
ISO 15223-1:2021 5.3.9		ISO 7000-2621	Atmospheric pressure limitation	Indicates the range of atmospheric pressure to which the medical device can be safely exposed.
ISO 15223-1:2021 5.4.2		ISO 7000-1051	Do not reuse	Indicates a medical device that is intended for one single use only.
ISO 15223-1:2021 5.4.3		ISO 7000-1641	Consult instructions for use	Indicates that the user needs to consult the instructions for use.
ISO 15223-1:2021 5.5.1		N/A	In Vitro diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
IVD Regulation 2017/746/EU		Annex I, Chapter III, 20.1h	Device for near-patient testing	Indication of near-patient testing
IVD Regulation 2017/746/EU		Annex I, Chapter III, 20.1h	Device not for self-testing	Explicit exclusion for assays not intended for self-testing or near-patient testing
IEC 60601-1:2005 +AMD1:2012 +AMD2:2020		IEC 60417-5032	Alternating current	Indicates that the equipment is suitable for alternating current only.

Standard/ Source	Symbol	ISO/IEC symbol number	Title of Symbol	Description of Symbol Per Standard
IEC 60601-1:2005 +AMD1:2012 +AMD2:2020		IEC 60417-5032	Alternating current	Indicates that the equipment is suitable for alternating current only.
IEC 60601-1:2005 +AMD1:2012 +AMD2:2020		ISO 7010-W001	General warning sign	To signify a general warning
Waste from Electrical and Electronic Equipment (WEEE) Directive		IEC 60417-6414	Waste Electrical and Electronic Equipment	Indicates that separate collection for waste electric and electronic equipment is required.
N/A		N/A	Illuminated Momentary Power Button	Indicates the power button on the back of the device. (Illuminated – Device is on / Light off – Device is off)
N/A		N/A	Saliva Drop Well indicator	Indicates where the user is to drop the saliva into the cassette.
N/A		N/A	CE Marking; European Conformity	Indicates that the manufacturer of the product complies with EU legislation and may be sold anywhere in the EEA (European Economic Area).

Questions or Concerns?

If you have questions or concerns regarding these instructions for use, please contact your Vigilant Biosciences Sales Representative or Vigilant Biosciences at customerservice@vigilantbiosciences.com.
For additional product information, visit www.vigilantbiosciences.com
If any serious incident occurs in relation to the device, please report to Vigilant Biosciences, US Food & Drug Administration, and/or the competent authority of the Member State in which the user and/or the patient is established.

REFERENCES

1. "The Science of Earlier: Improving early detection of oral and oropharyngeal cancer", White Paper, 2018, Vigilant Biosciences, Inc.
2. World Health Organization website. Accessed 2021.
3. Siegel RL, Miller KD, and Jemal A. Cancer statistics, 2019 CA Cancer J Clin 2019;69:7-34.

IVD For in *vitro* diagnostic use only.

PATIENT DATA PRIVACY

Vigilant Biosciences respects the privacy rights of individuals and is committed to handling and protecting personal information in compliance with the EU-U.S. and Swiss-U.S. Privacy Shield Frameworks. For our full Privacy Policy, visit our website at www.vigilantbiosciences.com. For complaints or concerns, contact our Privacy Administrator at privacy@vigilantbiosciences.com.

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Revision History

Document Revision	Description of Change	Effective Date
A	Initial Release	16-Apr-2021
B	Change in collection method	12-May-2021
C	Change in Authorized Representative	18-Apr-2022
D	Change in procedure to reflect product improvements	24-May-2022
E	Renaming of Device & Improvised Procedure Illustrations, Formatting correction, Warning addition	21-Mar-2023
F	Removal of duplicated warning, addition of Human serum albumin, coffee, mouthwash, toothpaste to a warning	28-Mar-2023
G	Update Age to Year of Birth, Addition of 'i' icon, Update to warning and precaution, Replace Wizard with OraFusion Software	26-APR-2023

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